June 2002 • NREL/MP-510-32388

Biofuels Program Semiannual Report

First Half FY2002
October 1 through March 31

R. Wooley

National Renewable Energy Laboratory

1617 Cole Boulevard
Golden, Colorado 80401-3393

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Prepared under Task No. BFP2-A101

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Acronyms and Abbreviations

AFUF	Alternative Fuels User Facility
ARC	American Radiolabeled Compounds
ARS	Agricultural Research Service
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
B/MAP	Biomass Agri-Products
BCA	bicinchoninic acid
BCI	BC International
BDT	billion dry tons
BFDP	Biomass Feedstock Development Program
BMG	Bateman Merrick Group
CAFI	Consortium for Applied Fundamentals and Innovation
CBS	Centraalbureau voor Schimmelcultures
CEC	California Energy Commission
CRA	Corn Refiners Association
CRADA	Cooperative Research and Development Agreement
DACS	data acquisition and control system
DDG	distiller's dry grain
DDGS	dry distillers grain with solubles
DE	directed evolution
DG	distiller's grain
DOE	U.S. Department of Energy
DSA	diafiltration saccharification assay
EERE	Energy Efficiency and Renewable Energy
EPAC	Ethanol Producers and Consumers
ES&H	environmental safety and health
FTIR	Fourier transform infrared
GC	gas chromatography
GCI	Genencor International
GIS	geographic information system
h	hour
HPLC	high-performance liquid chromatograph
HTS	high throughput screening
IEF	isoelectric focusing
LAP	laboratory analytical protocol
LCA	life-cycle analysis
LD	lignin depolymerase
MD	molecular dynamics
MESP	minimum ethanol selling price
MM	molecular modeling
MSDS	material safety data sheet
MTA	material transfer agreement
MTBE	methyl tertiary butyl ether

NCAUR	National Center for Agricultural Utilization and Research
NCGA	National Corn Growers Association
NDA	non-disclosure agreement
NIR	near infrared spectroscopy
NREL	National Renewable Energy Laboratory
NRRL	Northern Regional Research Laboratory
NYSERDA	New York State Energy Research and Development Authority
OHVT	Office of Heavy Vehicle Technologies
OIT	Office of Industrial Technologies
ORNL	Oak Ridge National Laboratory
PDU	Process Development Unit
PLA	polylactic acid
PLS	partial least squares
PNNL	Pacific Northwest National Laboratory
QA/QC	quality assurance/quality control
R&D	research and development
RFA	Renewable Fuels Association
SEC	size exclusion chromatography
SOP	standard operating procedure
SSF	SSF simultaneous saccrification and fermentation
TPI	total project investment
UPR	University of Puerto Rico
USDA	U.S. Department of Agriculture

COMMERCIAL PROJECTS

Summary of Technical Achievements or Results

All major tasks outlined in the Broin cooperative research and development agreement (CRADA) were completed on schedule. A meeting was held between Broin & Associates and the National Renewable Energy Laboratory (NREL) on March 7th to discuss the progress to date and next steps for this CRADA.

NREL developed FY2002 statements of work for the Distiller's Grain (DG) Conversion and the Advanced Corn Mills – U.S. Department of Agriculture (USDA) Partners Projects after consultation with two dry corn-milling ethanol operators and researchers at the USDA Agricultural Research Service(ARS) Eastern Regional Research Center and National Center for Agricultural Utilization and Research (NCAUR).

A preliminary process economic model for DG conversion was developed. The simulation results showed that converting DG into ethanol and animal feed co-product provides a possible option for improving the profitability of dry milling ethanol plants in light of continued decline in value of dry distiller's grain with solubles (DDGS).

General Technical/Scientific Progress

Broin CRADA

Milestone Progress/Completion

NREL will review the CRADA results with Broin before making recommendations for the next development activities.

Progress Highlights and Issues

All three major tasks were completed and documentation of experimental results is in progress. The CRADA period of performance was extended to finish the work originally planned.

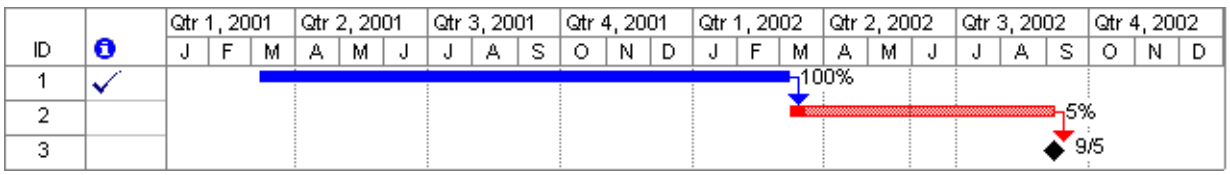

Figure 1. Broin CRADA
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Distillers Grain Conversion Project

Milestone Progress/Completion

C Milestone #378, Dilute-Acid Pretreatment Methods for Distillers Grain (9/30/02).
We expect to complete this milestone on time.

P Milestone #432, Corn Fiber Project Gate 2 Review (3/31/02).
The gate 2 review is scheduled for 4/17/02. Two industrial stakeholders have confirmed participation as gate reviewers.

Progress Highlights and Issues

A conceptual block flow diagram for the conversion of distiller's grain to ethanol was developed. Preliminary process economic evaluation looks promising. For a typical 25-million gal/yr dry mill, the maximum theoretical ethanol production from DG is about 5.0 million gal/yr. The ethanol production for a base case (where 92% of all hexose and 0% of pentose in DG are converted to ethanol) is 3.1 million gal/yr, and 4.7 million gal/yr for an improved case (where 92% of the hexose and 80% of the pentose is converted). The estimated minimum selling prices of ethanol for the base case and improved case are $0.80/gal and $0.52/gal respectively. The key assumption is that the value of protein (as animal feed) remains the same as in the original DG. It is possible that the pretreatment process could degrade the protein in the DG; however, it is also possible that the protein value may be improved through the secondary fermentation. Discussion with industry and animal nutritionists is under way to seek their participation in evaluating solid hydrolyzed DG as animal feed supplement.

NREL collected DG samples from two different corn dry mills, one in Nebraska and one in Minnesota. The chemical compositions of the samples are very similar. Three more samples are being analyzed to complete the preliminary survey of various dry mills and to identify any significant variation in chemical composition of DG. Preliminary results from steam pretreatment (with and without acid catalysis) of DG using the NREL 4-L steam reactor indicate that a strong acid catalyst is probably required to effectively hydrolyze most of the hemicellulose.

The traditional acid soaking method applied to lignocellulosic feedstock such as wood chips and corn stover does not work well for distiller's grain, as a significant amount of soluble material (e.g., starch, oil, and protein) is leached out during the soaking process. Modifications to bench-scale acid–impregnation and pretreatment equipment and procedures for processing DG are in progress.

Standard methods for analyzing solid composition of lignocellulosic feedstock are being re-evaluated for their applicability in high-starch and high-protein materials such as DG. Determining values for acid-insoluble lignin and acid-soluble lignin is of particular interest. These values appear to be high using standard methods because of possible interaction between protein and extractives (such as oil).

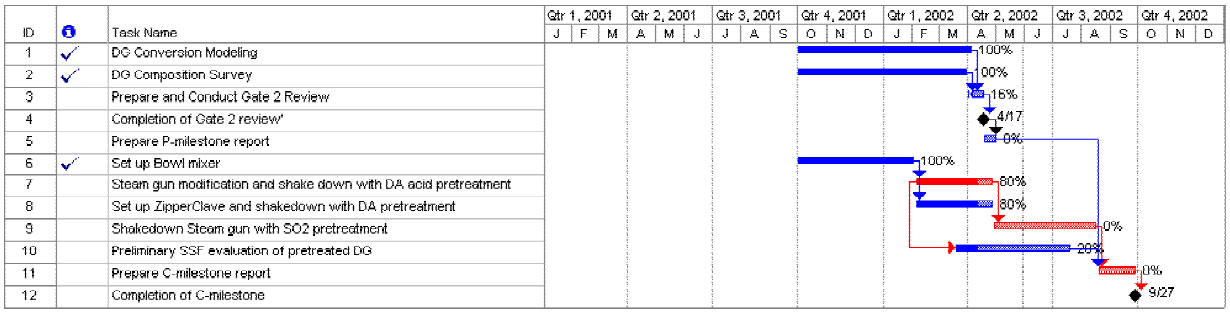

Figure 2. Distillers Grain Conversion Project
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Advanced Corn Mills Project

Milestone Progress

C Milestone #364 Pretreatment of Quick Fiber (8/30/02). This milestone is expected to be completed on time.

P Milestone #365 Gate 2 Review of Advanced Corn Mill Project (4/30/02). A gate 2 review is expected to be completed by 4/30/02.

Progress Highlights and Issues

The University of Illinois agreed to generate 20 kg of Quick Fiber for pretreatment experimentation at NREL. The pretreated materials will be sent to NCAUR and the Eastern Regional Research Center for further evaluations. Because of the high cost involved with producing a large amount of Quick Fiber, initial shakedown of pretreatment equipment will be carried out using corn fiber from a wet mill, which has characteristics and composition similar to the Quick Fiber.

As in the case with DG pretreatment, Quick Fiber requires us to develop new methods for dilute acid pretreatment and possibly new analytical methods. Given the limited time and budget, new analytical methods are unlikely to be developed in FY2002.

Scientific Publications, Presentations, and Other Activities
General Presentations/Travel

Quang Nguyen and Kelly Ibsen presented highlights of the Broin CRADA and DG Conversion results to representatives of Broin & Associates on March 7 at NREL.

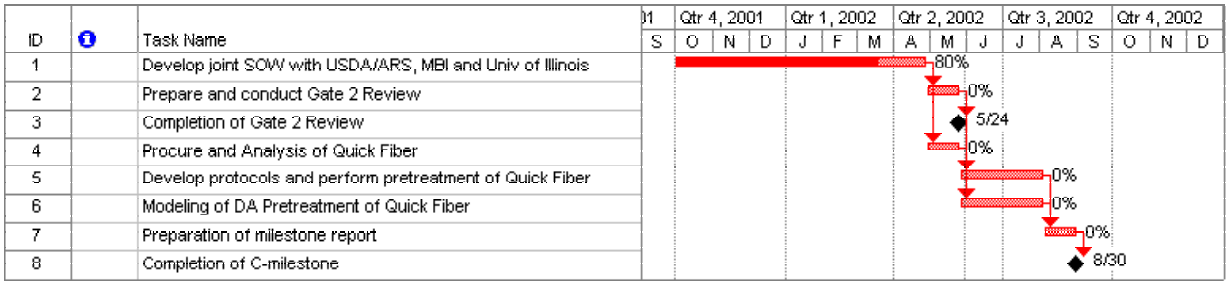

Figure 3. Advanced Corn Mills Project
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Summary of Technical Achievements or Results

Two-Stage Dilute Acid Project - Sealaska

A gate review was held and the results of the review showed the project to be economically unattractive. The project was put on indefinite hold while other technologies are researched.

Sealaska Corporation selected Ward Cove (near Ketchikan, AK) as the site for further study into building a demonstration plant for converting softwood residues to ethanol, and hired Bateman Merrick Group (BMG) of Aurora, CO to lead the development of a technical and business development plan.

TSS Consultants (Rancho Cordova, CA) performed a preliminary feedstock survey. They collected hog fuel and sawdust samples for analyzing composition and testing hydrolysis and determining the potential ethanol yield. NREL completed the Stage-2 hydrolysis experiments with the new feedstock. Concurrently, BMG and NREL developed a test plan for Stage-3 process development.

Based on the experimental results and cost evaluation, researchers decided that further investigation of alternate technologies would be required before selecting the most appropriate conversion technology for further development and demonstration.

General Technical/Scientific Progress

Milestone Progress

Stage-2 work activities identified in the Gate-3 Review (1/31/01) were completed. A meeting was held between Sealaska, BMG, the U.S. Department of Energy (DOE), and NREL to review the status of the Sealaska Bioenergy Project. It was decided that a Gate-3 review for the two-stage dilute acid hydrolysis process development plan is unnecessary. Based on the most recent business and technical information, the reviewers recommended that further investigation into alternate options for utilizing softwood residues before selecting the most appropriate technology for further development.

Progress Highlights and Issues

Feedstock Survey

TSS Consultants completed a new feedstock survey for a 300 billion dry tons (BDT)/day plant to be located at Ward Cove. Overall, a supply of about 500 BDT/day from surrounding areas was identified. In general, the feedstock quality from nearby sources is lower (i.e., more bark and less white wood) than was found in previous surveys. One of the reasons is that a portion of the feedstock comes from a local veneer mill, which generates bark-rich residues.

Stage-2 Work

Two-stage dilute acid hydrolysis experiments were carried out using two key feedstocks: Hemlock bark-rich hog fuel and Hemlock sawdust. Even though the process conditions were not optimized, the total glucose conversion was in the 46%-50% range. Sugar yield from the bark-rich material is low because of its high lignin and extractive content (about 59% w/w). The high bark content also appears to impact negatively on the sugar conversion.

Stage-3 Process Development Plan

BMG and NREL developed a work plan for bench-scale and pilot plant development of a softwood-to-ethanol process based on the two-stage dilute acid hydrolysis technology recommended in the "Bateman Preliminary Due Diligence Report" (1/15/01). This plan has been put on hold indefinitely.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

A meeting between representatives of Sealaska, BMG, DOE Golden Field Office (GO), DOE Office of Fuels Development (OFD), and NREL was held on November 28, 2001 to review the status of the Sealaska Bioenergy Project.

Scientific Journals: Paper Accepted for Publication

Kyoung-Heon Kim, Melvin Tucker, and Quang Nguyen, "Effects of Operating Parameters of Pilot-Scale Continuous Countercurrent Extractor on Recovery of Hemicellulosic Sugars from Pretreated Softwood". Accepted for publication in *Applied Biochemistry and Biotechnology*, Vol 91-93 (2001).

ENZYME SUGAR-ETHANOL PLATFORM

Summary of Technical Achievements or Results

During the reporting period, the Enzyme Sugar-Ethanol Project focused on finishing Stage 2 and beginning Stage 3 while continuing corn stover process development activities. The emphasis was on finishing the work necessary to complete Stage 2 and hold a Gate 3 project review. Major work areas were market assessment, techno-economic process modeling, life cycle analysis, pretreatment technology selection, and fermentation strain identification. Secondary emphases were on strengthening our ability to analyze corn stover feedstocks and process intermediates with higher accuracy and greater efficiency using rapid near infrared (NIR) spectroscopy-based methods, and on improving our understanding of the factors underlying compositional variance within corn stover. Limited experimentation was also carried out to produce representative process residue solids, to evaluate available pilot scale pretreatment capabilities, and to characterize and model enzymatic cellulose saccharification in the absence of simultaneous biomass sugar fermentation.

Two particularly important accomplishments were achieved during the reporting period:

- The Gate 3 review of the Enzyme Sugar-Ethanol Platform project, which represented a February 2002 K-milestone (#367), was completed in January—one month ahead of schedule. The review was held in Golden, Colorado on January 30-31 to determine if Stage 2 project work ("detailed investigation") had been completed satisfactorily and to critique plans for Stage 3 ("process development"). The review was extremely well attended, with over 50 external (non-NREL) representatives from industry, academia, and government participating. Attendees included 42 representatives from 25 companies and 4 universities, 3 representatives from USDA, 2 from ORNL, 4 from DOE/OFD, and 2 from DOE/GO. The distinguished panel of external reviewers was composed of Rod Fisher from Cargill, Dale Monceaux from Katzen International, Mel Pearson from Kvaerner, and Scott Nichols from Dupont.

While individual reviewer's comments differed, they unanimously agreed that the project was ready to begin initial Stage 3 work. A major concern of the external review panel was that the timeline for Stage 3 was too short. The reviewers believed that development/demonstration of the core pretreatment and enzymatic hydrolysis technology was of great value, but differed in their opinions regarding the appropriateness of the model feedstock (corn stover) and model product (ethanol) targeted for early Stage 3 process development activities. Reviewer and external participant comments have been consolidated and discussions are underway with DOE Biofuels Program management to decide how best to modify the project plan to respond to reviewer feedback. Results will be communicated early in the next reporting period.

Additional information about this project, including the presentations made at the Gate 3 review, are available at: http://www.ott.doe.gov/biofuels/enzyme_sugar_platform.html.

- Substantial progress was made in initiating a comprehensive survey of corn stover chemistry, chemical composition, and compositional variability. This work is essential to help define the "average" composition of corn stover and should elucidate the extent to which compositional variability is due to genetic and environmental parameters. More than 1,000 corn stover samples from the 2001 crop were obtained during the reporting period from about one dozen collaborators and contractors around the United States. Suppliers include academic institutions, agribusinesses, the USDA/ARS, and a corn germplasm collection maintained by the USDA. With the help of Hazen Research, Inc. (Golden, CO), all of these samples are being dried and milled in preparation for analysis by near infrared spectroscopy (NIR/PLS) to determine their chemical composition. NIR/PLS compositional analysis has already been started on the highest priority sets of samples, and we are on track to complete the associated July 2002 C-milestone on schedule. Plans are also being made to collect corn stover samples from the 2002 crop and thereby enable this critical study to be continued.

General Technical or Scientific Progress

Milestone Progress/Completion

K Milestone #367, Enzyme Sugar-Ethanol Platform Gate 3 Review (2/28/02)
The Gate 3 project review was successfully completed 1/31/02, one month ahead of schedule. The review was carried out following the Biofuels Program's stage gate methodology and included four industrial stakeholders as external reviewers. The external reviewers noted that some high-level issues needed to be better defined, most notably the project timeline, but nonetheless were impressed with the breadth and quality of the work completed in Stage 2 and recommended moving the project into Stage 3.

P Milestone #435, Confirm Pretreatment System Readiness (2/28/02)
The milestone was completed on schedule and documents the readiness or near-readiness of two pilot scale pretreatment systems—the smaller horizontal Sunds and larger vertical Sunds—to support work required to meet the Enzyme Sugar-Ethanol Platform Project's September 2002 pretreatment response surface C-milestone (#371). The horizontal reactor is the preferred system because of its ability to potentially achieve higher solids, higher temperature, and shorter residence time. Work to modify this reactor is underway and will be completed at the beginning of the next performance period, and a decision will be made on which reactor system to use for the response surface modeling. In the event that neither NREL reactor system is workable, then an outside organization with sulfuric acid pretreatment capabilities may be considered to support this effort.

P Milestone #434, Update Corn Stover Standard Laboratory Analytical Protocols (LAPs) (3/31/02)
This milestone was completed on schedule. Some of the revised LAPs specific for the complete compositional analysis of corn stover feedstocks and process intermediates are in final editing stage.
1) stover hydrolysis (new)
2) total solids
3) structural inorganics
4) carbohydrates
5) lignin

Others are still being modified or drafted but should be completed by late March.
1) extractives (modified)
2) protein (new)
3) uronic acids (new)
4) acetyl/formyl
5) total mass closure (modified to include the other nine procedures).

C Milestone #368, Develop Kinetic Model for Batch Enzymatic Saccharification to Enable Future Integrated Process Optimization (4/30/02)
This milestone is in progress and on track to be completed on schedule. The deliverable will be a report documenting completion of a preliminary operational kinetic model for stand-alone enzymatic cellulose saccharification—i.e., enzymatic cellulose hydrolysis in the absence of simultaneous fermentation—which incorporates enzyme adsorption, sugar product feedback inhibition, and temperature dependence over expected process relevant ranges.

P Milestone #433, Strategy for Industrial Involvement in the Enzyme Sugar-Ethanol Platform (4/30/02)
This milestone is in progress and on track to be completed on schedule. The deliverable will be a report documenting proposed strategies for industrial involvement incorporating recommendations of the sugar platform colloquy participants and DOE Biofuels Program management and describing constraints to government-supported deployment/commercialization activities.

P Milestone #436, Status Report on Corn Stover-to-Ethanol Life Cycle Analysis Modeling (5/30/02)
The objective of this milestone is to document refinements to Life Cycle Analysis (LCA) modeling methodology and results-to-date, and to discuss implications and make recommendations for what next steps should be taken to further improve the LCA's rigor, applicability, and visibility. The milestone will discuss the peer review input received to date (after presenting preliminary and revised LCA results in a number of forums in late FY2001 and the first half of FY2002) and propose a road map for moving forward with the LCA modeling work.

C Milestone #373, Summarize Current Understanding of How Genetic and Environmental Influences on Variability in Corn Stover Composition Affect Process Economics (7/30/02)

This milestone is in progress and on track to be completed on schedule. The deliverable will be a report documenting our understanding of how genetic and environmental factors influence corn stover composition and conversion characteristics, and the impact that this has on estimated process economics.

P Milestone #366, Develop Detailed Assessment of the Market and Pricing for Corn-based Ethanol During the Period 2002-2010 (7/30/02)
This milestone work is in progress and on track to be completed on schedule. The deliverable will be a report that will subsequently be sent out for external review. We anticipate that feedback from external reviews will enable Enzyme Sugar Platform Project researchers and biofuels program managers to understand the projected market for ethanol in the next decade, the pricing of corn-based and cellulosic ethanol under different scenarios, and estimate the optimal "market target" for ethanol product resulting from the Enzyme Sugar Platform Project.

C Milestone #371, Characterize Pretreatment Performance Response Surface (9/30/02)
Work in support of this milestone is just beginning, but no problems are anticipated in meeting the schedule. This said, it will not be clear for several more months whether the extensive experimental response surface work will be carried out in the larger vertical or in the smaller horizontal Sunds reactor. The milestone objective is to determine a response surface of pretreatment performance for the technology selected for the Enzyme Sugar Platform Project's initial Stage 3 work; dilute sulfuric acid pretreatment was the technology selected for initial Stage 3 work. We anticipate that the horizontal pretreatment reactor (see P-milestone #435) will be used to generate the response surface data and that this equipment should be able to operate at commercially relevant conditions. In particular, we would like to be able to explore pretreatment performance at higher solids loading ($\geq$ 30%), higher temperatures, and at shorter residence times than can be achieved in the larger vertical Sunds pretreatment reactor, since these conditions are predicted from kinetic modeling and previous experimental work in minipilot steam gun reactors to be capable of producing hemicellulosic sugar yields exceeding our Stage 3 working target of 85%. However, if the horizontal reactor cannot be used, we will re-evaluate all options available to us in an effort to meet the milestone objective.

P Milestone #438, Select Fermentation Strains for Further Stage 3 Testing (9/30/02)
Work towards completing this milestone will start upon receipt of candidate fermentation strains—anticipated in the 3rd quarter of FY2002. To date, only work using *Zymomonas mobilis* controls has been performed. We negotiated and established non-disclosure agreements (NDAs) with both Agrol (UK) and Purdue University to obtain access to their strains (*Bacillus stearothermophilus* and a Saccharomyces species, respectively), and will commence work once the strains arrive. Work scope has decreased, however, since these are the only strains that will be evaluated in FY2002 (beyond *Zymomonas mobilis* controls). Ethanologenic *Escherichia coli* was identified in Stage 2 as the strain with the highest potential. Negotiations are continuing with BC International (BCI) to allow Biofuels researchers to test their *Escherichia coli* (or *Klebiella oxytoca*) strains in the comparative screening

work. Alternatively, Biofuels researchers could observe fermentations carried out by BCI using hydrolyzates supplied by the Biofuels Program.

Progress Highlights and Issues

Stage 2 Progress and Findings

The objective of the Enzyme Sugar-Ethanol Platform project is to demonstrate economically attractive technology for producing ethanol from abundant lignocellulosic agricultural residues (corn stover is the model feedstock). The core process will build on existing pretreatment and fermentation strain technologies in combination with the next generation, lower-cost cellulases now being developed by Genencor and Novozymes. Project success requires breakthroughs in producing cellulases and in demonstrating commercially viable pretreatment and enzymatic cellulose hydrolysis conversion technology. The project is being managed using the Stage Gate project management framework. The goal of the Gate 3 review was to determine if Stage 2 (detailed investigation) had been successfully completed and, if so, to review and critique the plans for Stage 3.

The Stage 2 market assessment work showed that there is considerable uncertainty in the future ethanol market but suggested that the market opportunity is favorable provided ethanol can be produced at cost of $1.10/gallon or less. There is potentially a multi-billion gallon annual market for ethanol as a blend stock for gasoline at the proposed minimum ethanol selling price (MESP) target of $1.10/gallon, assuming the federal ethanol subsidy continues. A conceptual process and economic model based on continuous dilute acid pretreatment and hybrid hydrolysis and fermentation was used to estimate process costs and identify key parameter sensitivities. The model assumes that corn stover is available for $35/dry ton, that lower cost cellulases are available (i.e., enzyme development is successful), and that the integrated process performs at target levels believed to be achievable by 2005. The MESP is estimated to be $1.30/gallon for a plant processing 2000 dry tons of corn stover per day to produce 60 million gallons of ethanol per year. The capital equipment cost is estimated to be $115 million, which translates into a total project investment (TPI) of $200 million, or a TPI per annual gallon above $3. Sensitivity analysis results indicate that access to lower cost feedstock and the ability to co-locate the plant or obtain financing incentives for it are strategies that can be used to reduce the MESP by the additional $0.20/gallon needed to reach the proposed market target of $1.10/gallon. Even assuming that the MESP is attractive, however, the plant could have difficulty competing for financing with dry mill starch ethanol plants that have TPI/annual gallon values near $1.25.

Stage 2 work found no technical showstoppers to enzymatically converting corn stover to ethanol, but several areas were identified where important knowledge gaps remain that need to be addressed in Stage 3. In particular, more needs to be understood about the amount of corn stover that can be removed while maintaining soil quality, and about the potential for proposed advanced collection methods such as the so-called “one-pass whole stalk harvest” to reduce the cost (and increase the quality) of delivered corn stover. Knowledge gaps also remain in corn stover composition

analysis. Although substantial progress has been made in this area, further work to improve the analytical methods used for corn stover compositional analysis is needed in Stage 3 to permit process development to proceed in an efficient manner.

There are many technology development options, and key aspects—such as the temperature and pH range of the yet-to-be-developed cellulase enzymes—aren't yet known. A significant effort was made in Stage 2 to evaluate the numerous existing pretreatment technologies and fermentation strains and recommend a select few for experimental evaluation in Stage 3. Dilute sulfuric acid pretreatment was identified as the only pretreatment ready for Stage 3 process development, and demonstrating performance at 30% or higher solids level was identified as a key Stage 3 objective. (Several other pretreatments under active development also appear to have potential, but will only be considered for evaluation in Stage 3 if they can be shown to meet the project's readiness criteria.) Four ethanologenic biomass sugar co-fermenting strains were identified as the top candidates for Stage 3 (*E. coli* KO11, *S. cerevisiae* 424a [LNH-ST*], B. stearothermophilus* LLD-16, and *Z. mobilis* AX101 [as a control]). Comparative screening on corn stover pretreatment hydrolyzates needs to be performed in Stage 3 to rank strain performance.

The overall conclusions of Stage 2 work were positive and suggested that the project was ready to proceed into Stage 3. This said, the project remains a high-risk effort, and further work in Stage 3 will be required to validate many of the assumptions made in Stage 2. Stage 3 was initially envisioned to be a 3-year effort, but is likely to be extended based on external reviewer comments received at the Gate 3 project review. Technology options will be experimentally screened in year one to select a few promising systems for extended integrated process development work in year two and beyond. As reflected by the attendance at the Gate 3 review, industry is extensively involved in guiding the Stage 3 work.

The project has many tasks that must be executed in parallel. It moreover requires close coordination between several different project participants to be successful (e.g., DOE, NREL, ORNL, USDA, Genencor, and Novozymes). Beyond the need for effective coordination, critical success factors include the existence of fuel ethanol markets, the availability of low cost feedstock, access to cost-effective enzymes, and the ability to demonstrate robust integrated performance commensurate with compelling economics. Project success also requires continued adequate funding. Finally, high-level ownership of this project by DOE and USDA within the framework of the intergovernmental Bioenergy and Biobased Products Initiative is essential for the project to succeed.

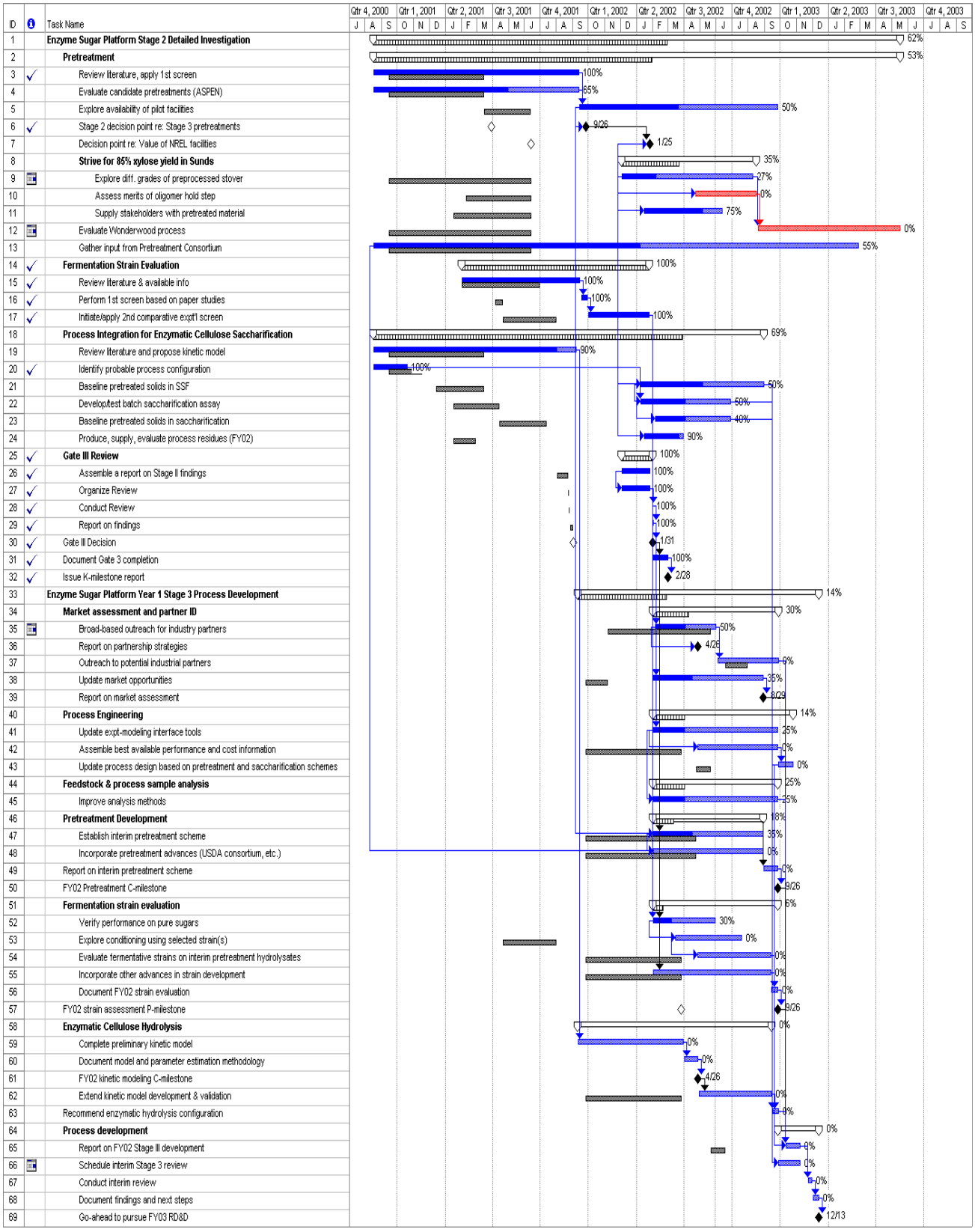

Figure 4. Enzyme Sugar-Ethanol Platform
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Life Cycle Analysis of Corn Stover-to-Ethanol

Preliminary results of the life cycle analysis of converting corn stover to ethanol were reported in a number of forums, including the Gate 3 project review, where feedback was actively solicited. In giving the green light for the start of Stage 3 activities, the Gate 3 reviewers stressed the importance of using LCA both as a tool to better understand the impacts and feasibility of stover as a feedstock for ethanol as well as a means of promoting dialogue with and outreach to the stakeholder community.

Based on the feedback received to date, we have been conducting additional LCA modeling work using the USDA's Century model for soil carbon effects and ORNL's geographic information system (GIS) model for corn stover collection and transportation. New Century model results and new GIS results were incorporated in a completely revised life cycle model for corn stover-derived ethanol during the reporting period, and NREL prepared a draft report and is soliciting detailed peer input. The new life cycle model for corn stover-derived ethanol is much more advanced than the preliminary model completed last year. The revised model allows for robust sensitivity analyses to examine how the rate of corn stover removal effects soil carbon levels, soil carbon fluxes, and corn stover collection and cost. In addition, we have expanded the system boundary used in the modeling methodology to include farm activities such as fertilizer application. This change is motivated by the need to look at changes in crop rotation patterns as part of the strategy to achieve sustainable corn stover collection. In particular, we are modeling to understand the differences between corn-soybean rotations and continuous production of corn. The model now accounts for increased fertilizer use in continuous corn production versus corn-soybean rotations (since soybean production requires almost no nitrogen fertilizer, while corn uses substantial amounts of nitrogen fertilizer). Fossil energy and greenhouse gas emissions are substantially lower for corn stover-derived ethanol, compared to corn starch-derived ethanol.

There are several important issues that have been raised by the revised model. For example, the new results suggest that on farms that operate with a corn-soybean rotation, collecting corn stover may not be possible under current conditions. This is because the model predicts that soil carbon levels will be flat or slightly declining over the next several decades, even without corn stover collection, in fields undergoing this type of rotation. Hence, removing corn stover from fields undergoing a corn-soybean rotation would only make things worse. On the other hand, the model predicts that switching to continuous corn production will allow corn stover to be collected while still maintaining soil carbon levels. However, it may not be practical or reasonable to suggest a price for corn stover based on continuous corn production. The next steps in exploring this issue will be to use the model to examine alternatives to continuous corn production that permit corn stover removal while maintaining soil carbon. The results of these sensitivity analyses are likely to have important ramifications for future research at USDA, which is the agency chartered with taking the lead on feedstock-related research on lignocellulosic ethanol production beginning next year.

During April and May 2002, we plan to gather feedback on the substantially revised study's results and also to use the model's results to re-assess research needs and

directions for the Enzyme Sugar Platform project and supporting feedstock infrastructure development work. Results, peer feedback, and recommendations developed through May 2002 will be documented in the May P-milestone report (#436).

Process Engineering and Technology Selection

Development and analysis using revised process model. NREL completed key work supporting the Gate 3 review, including updating the process design case (including projected economics). The preliminary process and economic model was refined and updated to better reflect our best understanding regarding corn stover conversion to ethanol using dilute sulfuric acid prehydrolysis (pretreatment) in combination with enzymatic cellulose hydrolysis and biomass sugar cofermentation. The revised process case assumes performance levels that we believe can be achieved during Stage 3 process development, including 85% xylose yields in prehydrolysis, 30% total solids concentration in the prehydrolysis reactor, and ethanol fermentation yields for glucose, xylose, and arabinose of 92%, 85%, and 85%, respectively. Based on the process case assumptions, the MESP is $1.30/gal (based on a 10% internal rate of return and 100% equity financing) and the TPI is $200 million for a 2000 dry metric ton feedstock per day facility that would produce 60 million gallons of ethanol per year.

Single-parameter sensitivity analyses were conducted on most of the process variables to determine those which have the largest impact on process economics. Parameters identified to have the largest financial impact are feedstock cost, enzyme cost, pretreatment hemicellulose sugar yield, fermentation ethanol yield, and the cost of capital. Most importantly, techno-economic modeling results vividly illustrate that reducing enzyme cost to approximately $0.11/gallon (through the enzyme development subcontracts to Genencor and Novozymes) is critical to the success of this project. Results also showed that production plant size, feedstock composition, pretreatment reactor solids loading, and byproduct credits were also important to project economics, but to a lesser extent.

Exploration of preliminary pioneer plant scenarios. Another substantial process engineering effort that supported the Gate 3 review (and related K-milestone) was to develop preliminary economic estimates for several prospective technology deployment (business opportunity) scenarios. These scenarios were explored to understand the potential for obtaining an additional savings of approximately $0.20/gallon to bring the estimated MESP from $1.30/gallon to the project's proposed (working assumption) market price target of $1.10/gallon ethanol. This would allow the produced ethanol to be highly competitive in the future U.S. fuel market.

Preliminary economics were determined for co-locating a cellulosic ethanol plant with either an existing coal-fired power plant or a new (greenfield) dry mill ethanol plant. High-level modeling with optimistic assumptions predicted a $0.07/gal savings by co-location with a dry-mill and $0.12/gal savings by co-location with the power plant. Since neither scenario by itself achieved the cost target, additional reduction techniques were included. Several scenarios were shown to reach the cost target, such as power plant co-location with cheaper feedstock or dry-mill co-location with

improved project financing. Any strategy that greatly reduces capital costs within the process will enhance its viability also.

Pretreatment Technology Selection. Work to compare potential pretreatment technology options continued in support of the Stage 2 and Stage 3 pretreatment technology selection efforts. In cooperation with several technology developers, generic ASPEN Plus-based process simulation models were developed that provide a consistent basis for economically evaluating acid-based, alkali-based, and non-catalyzed thermochemical pretreatment processes. These models will be used to assess and compare the economics of alternatives to dilute acid pretreatment as more rigorous performance data for the other pretreatment options becomes available.

Corn Stover Compositional Variability and Quality Evaluation

The overall stover quality (or sugar-ethanol potential) of a feedstock is a function of its chemical composition and conversion characteristics. Work carried out previously has shown that the chemical composition of corn stover is quite variable; to a much greater extent than the composition of previously examined feedstock materials, particularly yellow poplar sawdust. Past results show that corn stover chemical composition can be remarkably variable from batch to batch. Obviously, one factor affecting the chemical composition of corn stover relative to hardwood sawdust is the presence of many different parts of the corn plant (leaves, stalks, and cobs) compared to hardwood material obtained from a de-leafed and debarked tree. Since feedstock chemical composition directly determines theoretical ethanol yield, process economics are dramatically influenced. In addition to variability in chemical composition, variability in conversion performance was convincingly demonstrated in FY2001. We currently assume that variability in conversion performance is a function of differences in cell wall architecture—i.e., how the components are assembled into cell walls and linked together—rather than different proportions between components.

Building a robust bioconversion process requires a better understanding of the effects of variation in feedstock composition and cell wall architecture. Moreover, if we can understand the causes underlying feedstock compositional variability, it may be possible to manage the feedstock production parameters to favor improved biomass conversion process economics. The causes of compositional variability and differences in cell wall architecture are probably a complex function of crop genetics, environmental conditions (local climate and agronomic practices), harvest timing, harvesting method, and/or storage strategies. We do not currently have a clear understanding of which of these parameters are the most important in controlling feedstock quality.

In order to assess the potential genetic and environmental effects upon corn stover composition, we are analyzing a diverse sampling of freshly harvested corn stover produced in the United States last summer. This survey of corn stover chemistry will help to define an “average” corn stover composition and allow us to understand the extent to which genetic and environmental parameters determine compositional variability. During the reporting period, over 1000 corn stover samples from the 2001 harvest were delivered to NREL and dried in preparation for milling and NIR/PLS

compositional analysis. Most of these samples represent commercial hybrids that have been produced in different geographic locations around the U.S. corn belt and elsewhere. We expect that analyzing these samples will generate useful information about how genetic and environmental factors control stover quality and conversion performance. The samples have been prioritized so that we can obtain as much information about genetic and environmental effects as possible this fiscal year. We are currently in the process of milling and taking spectral data from each sample. Once reliable compositional information for these samples is available (which requires performing some wet chemical analysis for samples with atypical spectral characteristics), we will perform a statistical analysis of variance on groups of samples to identify parameters that appear to be important in controlling stover quality. A subset of feedstock samples, representing a wide variety of chemical compositions, will be selected and subjected to pretreatment and digestibility testing. These data will allow us to characterize how genetic and environmental factors influence conversion efficiency (see C milestone #373). Plans are also being made for harvest year 2002 stover collections.

Since the genetic relationships among commercial hybrids are generally not publicly available, we are relying on information from corn seed companies and corn breeders that have provided stover samples to help us identify a subset of varieties that represent a wide range of genetic diversity. To this end, we have initiated discussions with three major corn seed companies and intend to broaden these interactions during the next reporting period to include additional companies and academic corn breeders.

Although it is widely accepted that there is significant mass loss from corn stover bales during outdoor storage, previous work has shown no dramatic change, on a dry weight basis, in the chemical composition of corn stover during prolonged outdoor storage. In particular, previous studies show less variation during storage than is observed between different corn stover samples. Thus, research into changes due to long-term storage has been de-emphasized this year. Rather, we are working with ORNL and searching the literature to begin to quantify typical mass losses during storage.

One of the goals of this activity is to strengthen the working relationships between researchers in ORNL's Biomass Feedstock Development Program (BFDP) and at NREL. To this end, S. Thomas attended a BFDP subcontractors' workshop in Memphis, TN, in November 2001. J. McMillan, S. Thomas, J. Sheehan, and D. Templeton (all NREL) are also in contact with S. Sokhansanj, J. Cushman, and L. Wright (all ORNL) on a regular basis to discuss issues related to corn stover feedstock logistics.

Surveying the technical literature and attending scientific meetings during the past year, with special attention to cell wall biogenesis, composition, and architecture has revealed many possibilities for genetic modification of the quality of corn stover. An ever increasing and somewhat bewildering array of genes has been described that influence the synthesis of each of the major cell wall polymers. Using the techniques of classical genetics, genomics, bioinformatics, and genetic engineering, labs around the world have been able to significantly change both lignin quantity and quality in

plant cell walls from a variety of species. An indication that these sorts of genetic changes might alter biomass processing strategies and economics is given by the fact that some of these changes have been shown to impact pulp and paper making processes. In addition, the large plant biotechnology companies are actively looking at manipulating cell wall chemistry to enhance the ruminant digestibility of corn forage/silage. Cellulose synthase genes from several species, including corn, rice, and *Populus* spp., have been cloned and sequenced. The availability of these genes (and mutations in some of them) makes the option of increasing, decreasing, or altering the proportion (and possibly the relative crystallinity) of cellulose in plant cell walls feasible. Clever mutant screens and the characterization of cellulose synthase genes have also been instrumental in identifying genes encoding glycosyl transferases that are proposed to be involved in hemicellulose biosynthesis. A different glycosyl transferase is probably required for every sugar and glycosidic linkage formed in the biosynthesis of hemicellulose.

In December 2001, the Biofuels Program hosted a 4-hour plant cell wall mini-course taught by two well known investigators in the field of plant cell wall biosynthesis, Drs. Maureen McCann (John Innes Centre, Norwich, England) and Nick Carpita (Purdue University). Approximately 30 Bioenergy Center staff attended, along with scientists from the University of Colorado, Colorado State University, and Hazen Research, Inc. The course was extremely well received by all, and we hope to reprise the effort this spring.

A working relationship (established in FY2001) with Monsanto scientists who are responsible for biomass conversion-related business opportunities and R&D is also continuing. This effort is responsible for an excellent subset of the samples being used in this year's stover quality research. We are also continuing a working relationship with researchers at Pioneer Hi-Bred that began in FY99. We recently established a promising relationship with Syngenta, another large agbiotech company with a significant presence in the corn seed industry. Relationships with smaller plant biotech companies, such as ProdiGene, Inc. (College Station, TX), Athenix (Durham, NC), and Ceres, Inc. (Malibu, CA) are also being actively maintained and cultivated.

Corn Stover Pretreatment

The focus of pretreatment work carried out during the performance period was to narrow the number of pretreatment technology options in advance of initiating more experimentally intensive Stage 3 process development work. Progress was made in two areas in support of this goal. First, a Stage 2 technology review was completed to survey the performance, development, and demonstration status of potentially available pretreatment technologies. We solicited information directly from technology developers and also sorted through the copious information available in the public literature. The initial screening steps to identify promising pretreatment technologies were completed. Thirteen different pretreatment technologies were screened in detail to scope the Stage 3 technology development work phase of the project. As of January 2002, only the data on dilute acid pretreatment technology is sufficient to justify commencing Stage 3 work. However, additional performance data is expected to become available for several other pretreatments during the next reporting period.

Quantitative screening of alternative pretreatments will be performed as such data become available. The FY2003 plan for investigating and/or further demonstrating pretreatment will depend upon the data and process and economic modeling results generated during the second half of FY2002.

The second accomplishment during the reporting period was to experimentally evaluate the capabilities of dilute sulfuric acid pretreatment of corn stover using the Biofuels Program's larger continuous pilot scale (vertical) Sunds pretreatment reactor (located in the 1 ton/day Process Development Unit (PDU) at NREL's Alternative Fuels User Facility (AFUF). Understanding our current capabilities for performing dilute acid pretreatment of corn stover using available pilot scale equipment is critical to scoping the Stage 3 process development and determining if previously unrecognized issues are present when trying to pretreat corn stover under industrially relevant conditions (e.g., at substantially higher solids loadings than we worked with before using this feedstock). During the previous reporting period, corrosion damage to the agitator system in the exhaust "flash" tank halted this "benchmarking" work. Repairs were completed in the beginning of the current performance period and the repaired and improved system was operational in December 2001.

Since then, a number of additional pretreatment runs were carried out using 20% and higher solids concentrations using a new lot of corn stover supplied by Biomass Agri-Products (B/MAP). Significantly higher pretreatment hemicellulose sugar yields and enzymatic cellulose digestibilities were achieved using the new feedstock, and we are currently trying to understand the cause of the improved performance. Total xylose yields on the new batch of feedstock using the repaired system ranged from 75% to 81% with enzymatic cellulose conversion (using our standard simultaneous saccrification and fermentation (SSF) -based assay) ranging from 90% to 97%. These results compare to total xylose yields of 70%-73% and cellulose conversion of 80%-87% obtained previously using an earlier batch of corn stover feedstock. Also notable was the fact that we were able to operate the Sunds reactor at solids concentrations up to 28% with no apparent reduction in yields. These accomplishments are exciting and further work is planned to elucidate the causative factors underlying this substantially improved performance.

Based on what we know today, we hypothesize that the significant compositional differences in the two batches of feedstock may be contributing to the improved performance. In particular, on a dry weight basis the new material contains 7%-20% more carbohydrates (as glucose and xylose) and approximately 50% more acetate. Although unconfirmed at this time, we suspect that B/MAP's new washing and size reduction system may be affecting the composition of the feedstock by selectively changing the ratio of the different components (stalks, leaves, and cob) to a composition that is easier to convert. The corn variety and harvest method may also be causative factors for the compositional differences. We will be receiving more corn stover from B/MAP in the near future and will carry out tests during the next performance period to better understand this issue. Results of the feedstock composition study will be reported in the July C-milestone (#373) and are expected to contribute to our understanding of this issue.

Kinetic Modeling of Enzymatic Hydrolysis

Significant progress was made during the reporting period in mathematically modeling the enzymatic hydrolysis of cellulose in the absence of simultaneous fermentation. This is an extremely complicated system to model, since enzymatic hydrolysis of cellulose involves substrate heterogeneity and a multi-enzyme cellulase complex. Beyond this, there is uncertainty about the ultimate nature of the cellulases that will be developed by Genencor and Novozymes and key issues such as the process configuration and conditions under which these enzymes will be used. In addition, modeling is challenging because our ability to validate any model is confounded by what we can experimentally measure in terms of system performance and mechanistic behavior. These challenges notwithstanding, our ultimate goal is to develop a kinetic model that is sophisticated enough to be used for *in silico* process optimization.

A working kinetic model was substantially developed during the reporting period; documentation of this model represents an April 2002 C-milestone (#368). The preliminary model is grounded in the biochemistry of enzymatic hydrolysis. It incorporates adsorption of enzyme components onto cellulose surface structure, provides some structure in the enzyme system by distinguishing between the exo/endo glucanase and β-glucosidase components of the cellulase complex, and includes the effect of sugar end-product inhibition (which in contrast is mostly absent in an SSF system). Three hydrolysis reactions are modeled; two heterogeneous reactions for cellulose breakdown to cellobiose and glucose, respectively, and a homogeneous reaction for cellobiose to glucose conversion that occurs in solution. Cellulase adsorption onto pretreated biomass is modeled using a Langmuir-type isotherm, and sugar end-product inhibition is described using either noncompetitive or competitive models, and notably includes potential inhibition caused by xylose—the dominant sugar present in dilute acid hemicellulose hydrolyzates.

In total, the reaction and mass balance equations contain twelve independent parameters. Parameter estimation was performed using Matlab®. Briefly, a Matlab® optimization function was used to determine best-fit model parameters from shakeflask enzymatic cellulose saccharification data generated using dilute-acid pretreated corn stover substrate (nominal 10% solids loading, or ~6% by weight cellulose) and our commercial reference cellulase preparation. Only modest differences were noted in the model's predictive abilities using either noncompetitive or competitive end-product inhibition. Both approaches predicted hydrolysis performance well for the first 48 hours but overestimated glucose production rates thereafter. Consequently, the mathematically simpler noncompetitive model was used in subsequent simulations. The preliminary model describes product inhibition behavior to the extent that it satisfactorily predicts the effect of added β-glucosidase on corn stover hydrolysis at different background glucose levels. Not surprisingly, the best-fit model parameters are specific to pretreated corn stover. For example, the model overpredicts the initial kinetics observed in enzymatic saccharification of pretreated yellow poplar.

As mentioned above, a notable feature of the preliminary kinetic model is that it overpredicts glucose production during the later stages of saccharification. In other

words, the model is not able to adequately describe the decrease in cellulose hydrolysis rate as the extent of cellulose conversion increases and glucose accumulates towards the end of the process. Although one can speculate about the precise causes of this phenomenon, for modeling purposes a parameter that enables decreasing substrate reactivity was introduced in an attempt to improve the model's behavior. Washed residual substrates harvested at different times during the course of the hydrolytic process were saccharified using fresh buffer and fresh enzyme to minimize the potential for significant end-product inhibition or enzyme inactivation to confound measuring the actual decrease in hydrolysis rates. Based on the relative hydrolytic rates observed on residual cellulosic substrates obtained at different extents of conversion, a substrate reactivity-type index of the following form was postulated: $R = \alpha S/S_0$, where R represents the relative substrate reactivity, α is a substrate reactivity parameter, and S/S_0 is a dimensionless substrate concentration. The best-fit value for α is close to unity, so the correlation simplifies to $R = S/S_0$. We should point out that incorporating this parameter in essence renders the hydrolysis reactions to a second order with respect to cellulose concentration, and can also be used to incorporate other factors detrimentally impacting hydrolysis performance in a similar fashion.

Another feature the preliminary model incorporates is the ability to predict the effect of temperature on enzyme activation and deactivation over the expected process-relevant ranges. The challenge here, of course, is that we do not have thermostable enzymes to evaluate and so must make due with using currently available preparations. Based on saccharification data at temperatures of 30°C–65°C using our reference commercial cellulase enzyme, the temperature optimum for cellulose hydrolysis lies between 50°C and 60°C (most likely at about 55°C based on initial hydrolysis rates). Enzyme activation with respect to temperature using this enzyme appears linear, since a linear rather than exponential model best predicts experimental results obtained at higher temperatures. However, an exponential Arrhenius-type model is better for describing enzyme deactivation at temperatures above 60°C. These temperature effects are incorporated into the model.

Work performed in March and continuing into April has focused on model validation (comparing predictions made at conditions different than those used to fit the model's parameters with actual experimental data) in order to understand the predictive capabilities of the model. A key objective is to identify any critical limitations in the preliminary model that should be resolved in subsequent work. The preliminary model is specific to the substrate and enzyme system used and will need to be updated and refined as improvements are made in feedstock pretreatment and hydrolytic enzymes, and perhaps if substantial changes in feedstock composition or conversion characteristics occur.

The C milestone #368 report documenting kinetic model development is being prepared and will be completed on or ahead of schedule. The preliminary model should be useful for evaluating the initial stages of enzymatic saccharification when it is carried out using a hybrid hydrolysis and fermentation process configuration as part of an integrated corn stover-to-ethanol process.

Fermentation Strain Selection

Through literature review, initial screening of MESP cost reduction potential, and discussions with strain developers or license holders, three candidate strains were selected in Stage 2 for Stage 3 experimental process development. These strains include: Purdue University's recombinant *Saccharomyces cerevisiae* 424A LNH-ST; BCI's ethanologenic *Escherchia coli* strains KO11 and SL40; and Agrol Ltd.'s *Bacillus stearothermophilus* (*ldh* mutant). NREL's recombinant *Zymomonas mobilis* is also being included as a control, since this is the only type of strain for which we have already developed an integrated performance baseline (albeit on hardwood yellow poplar). The strain developers or owners/license holders for these strains were contacted to determine the availability of their strain(s) for third-party licensing and performance verification. Non-disclosure agreements were negotiated and signed with Agrol and Purdue University, and we are now waiting for these strains to arrive. Once the candidate strains are received, we will reproduce results reported by the strain developers to ensure to their satisfaction that we can handle their strains appropriately. After this is done, we will begin evaluating the strains on pretreated corn stover hydrolyzate liquors.

BCI chose not to supply a strain for the comparative performance screening work at NREL. Their *E. coli* strains remain available for third party licensing and BCI is willing to perform experiments observed by Biofuels Program researchers to verify their strain's performance.

Residue Production

During the second half of the reporting period, NREL carried out another large-scale residue production run. The objective was to produce sufficient material to supply residue to key stakeholders, especially the USDA who had requested >50 kg of residue (dry basis) for scaled up soil studies. This residue production run used pretreated corn stover that was produced over two days using the PDU's Sunds pretreatment reactor. The general SSF methodology used was that established previously in the smaller 1450-L fermentor scale during the preceding reporting period. In this large-scale run, the SSF residue was produced in one of the PDU's 9000-L fermentors using our reference commercial cellulase preparation and *S. cerevisiae* D5A as the fermentation microorganism. This run was completed in late February 2002 and produced a total of approximately 90 kg (dry basis) of washed solid lignin-rich residue. After compositional analysis is performed, we will ship out material (and an accompanying material safety data sheet (MSDS)) to external stakeholders at the USDA ARS (MN), Energy Efficiency and Renewable Energy (EERE) (ND), Novozymes Biotech (CA), and Pacific Northwest National Laboratory (PNNL) (WA). A small amount of material will be held back into order to supply other stakeholders who we expect will be contacting us during the next reporting period.

Subcontractors

Hettenhaus, Chief Executive Assistance
ACO-1-31042-01
"Innovative Methods for Collecting, Handling, and Transporting Corn Stover"
6/27./01 – 6/30/02 (no cost extension through 9/30/02)
CEA will investigate several innovative approaches to reducing the costs of corn stover collection and storage. A final report will be delivered to NREL that documents their analysis, including their preliminary estimates of the economics of the proposed approaches. Recommendations will be made regarding future work priorities that will help to further decrease the cost of delivered corn stover to a bioethanol or biorefinery processing plant.

Hettenhaus, Chief Executive Assistance
ACO-1-31108-01
6/5/01 – 9/30/02
Partnership Development Support with the Sugar Platform Industry
Cindy Riley is the Technical Monitor for this subcontract. The mod to extend the subcontract was recently awarded.

Curran, Biomass Agri-Products (B/MAP) LLC
XCO-1-31021-01
Prepare and Supply Corn Stover for Pretreatment and Bioconversion Testing
03/23/2001 - 06/29/2005 (FY02 dates 6/30/02-6/29/03)
The installation and shakedown of washing equipment was completed. B/MAP has since prepared several batches of washed corn stover in accordance with requested specifications (within the ability of their mechanical systems). This material is supporting Enzyme Sugar-Platform and Advanced Pretreatment project work, as well as CAFI work.

Agblevor, Virginia Polytechnic Institute & State University
Alternatives to Overliming
XCO-9-29049-01
5/24/99-9/30/02 (FY02 4/1/02 – 9/30/02)
There was a lag in initiating this work due to delays in getting staff hired. Work has since commenced, and the emphasis has switched from poplar hydrolyzates to corn stover hydrolyzates. We plan to re-compete this subcontract in FY03.

TBD
Life Cycle Analysis
TBD-02-31-A
Again, this subcontract is likely to placed late owing to delays/uncertainties in programmatic funding during the 1st half of the performance period. We are uncertain if this will be placed in FY02.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- J. McMillan presented an invited seminar entitled, "Commercializing Enzymatic Biomass Conversion Technologies: Opportunities for Biorefineries Producing Sugar, Ethanol, Lignin, and Power," at Oklahoma State University on October 16, 2001. While there, he toured their facilities and met with several university professors, researchers, and students involved in bioenergy and biobased products work.

- J. McMillan and K. Kadam attended the annual meeting of the American Institute of Chemical Engineers in Reno, Nevada, in November 2001, chairing technical sessions and presenting a co-authored poster (see below).

- J. McMillan visited Genencor and Novozymes in December 2001, presenting updates of the Enzyme Sugar-Ethanol Platform project and discussing cross project coordination needs with senior members of the enzyme development project teams at each of these companies.

- J. Sheehan and S. Thomas attended the ORNL/BFDP subcontractor's workshop in Memphis, TN, November 7-9, 2001. J. Sheehan gave a presentation which included a Biofuels Program overview and a discussion of important feedstock issues from a conversion perspective.

- S. Thomas visited Athenix, a start-up plant biotechnology company in Research Triangle Park, NC, on February 21-22, 2002. Thomas was invited to participate as an external technology expert in a videotaped set of interviews on the topic of alternative energy and renewable chemicals produced by WJMK Productions (Boca Raton, FL) that may air on PBS. Thomas also presented a seminar to the staff of Athenix on biomass conversion and participated in discussions with the staff.

- S. Thomas visited BASF's plant biotechnology R&D company in Research Triangle Park, NC, on February 25, 2002. BASF is a large German chemical company. He presented a seminar on biomass conversion to BASF staff members and answered questions on the topic.

- S. Thomas and J. Ashworth visited Syngenta's plant biotechnology R&D unit in Research Triangle Park, NC, on February 26, 2002. Staff and managers from Zymetrics also attended the meeting. Zymetrics is a wholly owned subsidiary of Syngenta that has access to technologies and microbial gene collections owned by Diversa (San Diego, CA). S. Thomas presented a seminar on biomass conversion, and the day was spent in discussion with staff scientists, business development, management and legal people. There appear to be significant mutual interests among these three organizations, and we will continue to explore them.

- S. Thomas and S. Bower attended an ORNL/OIT-organized workshop in Chicago, IL, on March 7, 2002, entitled “Identifying High Potential Crops and Bioproducts”.

- Steve Thomas will attend a small international meeting on plant cell walls at the UCLA Conference Center in Lake Arrowhead, CA, May 12-15, 2002.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- S. Thomas et al. submitted an abstract for a poster or short talk describing progress on understanding corn stover quality to be presented at the annual meeting of the American Society of Plant Biologists to be held in Denver, CO, August 3-7, 2002.

- Saccharification of lignocellulosic biomass in the context of a common platform for fermentable sugars by K.L. Kadam and J.D. McMillan. Poster presented at the AIChE Annual Meeting, Reno, NV, November 4-9, 2001.

- Five posters completely or partially relating to the Enzyme Sugar-Ethanol Platform project were presented as part of the American Institute of Chemical Engineers’ Critical Issues Series held March 10-12, 2002, at the Spring National Meeting in New Orleans, Louisiana:

    - “Realizing Lignocellulose Biorefineries: The Enzyme Sugar Platform Project” by J.D. McMillan

    - “Overview of the Planned Demonstration Facility for Enzymatic Hydrolysis of Biomass” by J. Ashworth

    - “Technical and Economic Feasibility Assessments in the DOE Biofuels Program” by A. Aden, K. Ibsen, and M. Ruth

    - “Rapid Biomass Analysis: New Analytical Methods Supporting Ethanol Production from Biomass” by B. Hames, et al.

    - “Life-Cycle Analysis of Ethanol from Corn Stover” by J. Sheehan

- Riley, C. J. “Bioethanol: A Renewable Transportation Fuel from Biomass.” Oral presentation at the American Institute of Chemical Engineers Spring National Meeting, New Orleans, LA, March 12, 2002.

- Riley, C. J. “Bioethanol: A Renewable Transportation Fuel from Biomass.” Oral presentation at the 11th Western Photosynthesis Conference, Monterey, CA, January 5, 2002.

General Technical or Scientific Progress

Enzyme Subcontract Liaison

The objective of this project is to ensure that the enzyme development subcontractors – Genencor International (GCI) and Novozymes Biotech (NB) – are provided with all of the technical information from the Biofuels Program's years of cellulase and bioethanol process development research in the most useful manner. A key role of this project is to ensure the quality and process relevance of the metrics and cellulase performance assays and assay results being used to benchmark and measure improvements in the cellulose hydrolysis efficiency of existing and novel cellulases and complete cellulase preparations. The results of selected performance comparisons are used to assess the progress being made in each subcontract with respect to enzyme cost targets as well as to help to guide their future research efforts. This project also serves as a conduit for communication and technical planning between DOE, various NREL projects, and each of the commercial enzyme developers (GCI and NB). Finally, this project is responsible for controlling and protecting the respective subcontractor subject and background data.

We continued to extensively interact and work with both enzyme developers during the performance period, providing both fundamental and process-relevant performance data on a number of cellulase samples from GCI and NB. We moved from using pretreated poplar sawdust as the reference cellulosic substrate in our standard enzymatic hydrolysis performance assays to using pretreated corn stover, and re-evaluated all of the benchmark cellulase enzymes using pretreated corn stover. This support work is ongoing and current work is directed at developing a new assay for evaluating the improved cellulase enzymes that will allow the enzymes to be evaluated under different enzymatic saccharification process configurations.

A highlight accomplishment was generating and supplying performance data to NB that was critical to confirming that they had met their first year cost reduction objective. As a consequence of this work, the first year target of NB's three-year subcontract, aimed at reducing by a factor of ten the cost of cellulases for enzymatic saccharification of cellulose, was successfully completed January 31, 2002. Using a variety of advanced biotechnology techniques, NB succeeded in achieving a 2.3 times reduction in the estimated cost of cellulases required for an enzyme-based bioethanol process. Biofuels Program research staff tested the performance and specific activity of NB's benchmark enzymes and were integral to verifying this achievement. The cellulase development and cost-reduction work will continue at an accelerated pace in the upcoming months, and will include Biofuels Program research staff testing the performance of the new improved cellulases.

Milestone Progress

K Milestone #431 Cellulase Cost Reduction (9/30/2002). Performance to be measured in closed-tube hydrolysis reaction and compared to Novozymes' Celluclast 1.5L product under standard reaction conditions (pH 5.0, 38°C, 72 h at 10% PCS loading).

Novozymes will provide enzymes with 4-fold improved performance in the hydrolysis of pre-treated corn stover. Milestone progress is on track.

Progress Highlights and Issues

The GCI and Novozymes subcontracts were monitored via bi-monthly meetings and reports. The NREL staff serving a subcontract monitors (Himmel and Nieves, respectively) are also coordinating testing and benchmarking activities to ensure efficient and equivalent support for both subcontractors.

Validation and/or "benchmarking" studies of cellulase samples provided by the subcontractor(s) to NREL were conducted. A number of cellulase samples have been received from GCI and Novozyme for testing at NREL. Upon receipt, a portion of each sample was desalted by means of a standard size exclusion chromatography (SEC) treatment and characterized in terms of protein content by the Micro (Pierce) bicinchoninic acid (BCA) assay. A number of diafiltration saccharification assay (DSA) experiments have been carried out on these samples, including, at minimum, duplicate or triplicate standard assays of each.

DSA loading-response curves were obtained for the performance of Genencor Spezyme in the saccharification of standard pretreated corn stover at 38^{o}C, pH 5.0. Two commercial cellulase preparations recommended by Novozymes were also compared for use in saccharifying pretreated corn stover at pH 5.0, 38^{o}C; these were Celluclast 1.5L and Cellubrix.

Subcontractors

Genencor International, C. Mitchinson
ZCO-1-30017-01
5/2000 – 7/2003
Cellulase Cost Reduction for Bioethanol
During this period Genencor International has continued to investigate improvements to various cellulases and cellulase production technologies. The project has focused on the expression of new cellulase genes for biochemical characterization. Genencor has identified a number of promising cellulase candidates with improved performance relative to the cellulases that are currently commercially available and are working on methods to produce the proteins economically. Genencor continues to focus on the improvement of cellulase specific activity, increased levels of production, and stability of the component enzymes. Additionally, new screening assays are being developed to improve the rapidity by which new enzymes are tested.

Novozymes Biotech, Inc., J. Cherry
ZCO-1-30017-02
Cellulase Cost Reduction for Bioethanol
12/19/00 - 6/19/04
During this period Novozymes Biotech has continued to investigate improvements to various cellulases and cellulase production technologies. The project has focused on the expression of new cellulase genes for biochemical characterization. Novozymes

has identified a number of promising cellulase candidates with improved performance relative to the cellulases that are currently commercially available and are working on methods to produce the proteins economically. Novozymes continues to focus on the improvement of cellulase specific activity, increased levels of production, and stability of the component enzymes. Additionally, new screening assays are being developed to improve the rapidity by which new enzymes are tested.

Scientific Publications, Presentations, and Other Activities
General Presentations/Travel

M. Himmel, R. Nieves and B. Wooley made presentations at planned bimonthly reviews and year-end meetings with GCI and Novozymes Biotechnology.

APPLIED RESEARCH

Summary of Technical Achievements or Results

Advanced Pretreatment

As the result of recent gate reviews and interim gate reviews for several pretreatment projects, the organizational structure of the Advanced Pretreatment Project has been changed. This new structure identifies four major work areas: 1) technology options evaluation and selection, 2) pretreatment reaction mechanism fundamentals, 3) pilot scale equipment capabilities development, and 4) hot wash process applications. This structure is reflected in the FY 2002 Biofuels Program Annual Operating Plan.

The Technology Options Evaluation and Selection subtask is working with several pretreatment technology providers, including those in the Biomass Refining CAFI and selected others. Efforts are underway to gather current and potential process performance data for the various pretreatment approaches and to develop ASPEN-based process engineering models for each pretreatment approach. This information is being utilized to develop criteria that will be used to identify pretreatment approaches with the potential of meeting advanced technology process performance and economic targets.

The Pretreatment Reaction Mechanism Fundamentals Subtask is continuing molecular and quantum modeling efforts to understand the structured water effects for thermochemical and enzymatic hydrolysis of cellulose. Recent modeling efforts have shown that significantly less structuring of water molecules occurs at the step-face surface of the cellulose micro-fibril as compared to the parallel face, where significant water structuring apparently exists. This suggests that the step face is most likely the preferred site of catalytic attack by the hydronium ion.

The Pilot Scale Equipment Capabilities Development Subtask is focusing efforts to verify short residence time and high solids loading capabilities in the recently reconfigured 200 kg/day horizontal pretreatment reactor system. Such capabilities may provide additional pilot-scale pretreatment system equipment options and a greater range of operating conditions than in the 1 ton/day Sunds hydrolyzer, especially for low bulk density feedstocks such as corn stover. Additionally, pre-installation activities are underway to support the installation of the pilot scale Pneumapress pressure belt filter in the PDU. This filter is expected to be delivered in early April 2002. The Pneumapress filter will be used for general solid-liquid separation operations in the PDU and to test the hot washing pretreatment concept in conjunction with the horizontal reactor system.

The Hot Wash Process Applications Subtask is gathering additional data on this concept to verify high xylose yields and to determine potential cellulase loading impacts. A key emphasis of this subtask is to fundamentally characterize the properties of soluble lignin fractions resulting from the hot wash process and to

provide representative samples of such fractions to interested industrial concerns so that they can determine if any attractive co-product opportunities are worth pursuing.

General Technical or Scientific Progress

Milestone Progress/Completion

K Milestone #409, Potential of Various Pretreatment Technologies to Meet 2010 Pretreatment Conversion Goals (8/31/02)
Current efforts to meet this milestone are focused on process information gathering and technoeconomic model development for various pretreatment technologies. Both of these efforts are being conducted in direct collaboration with leading technology providers for each pretreatment technology. This will be followed by the development of criteria to be used in determining which pretreatment technologies have the potential to meet advanced technology process performance and economic targets.

C Milestone #404, Boundary Layer Theory Stage A Interim Review (7/31/02)
Experimental data now suggests that the "shrinking bed" reactor's physical operation, when using native yellow poplar, facilitates some undefined chemistry, such that either added catalytic activity is manifested or that glucose-lignin chemistry (association or covalent bond formation) is altered. Molecular modeling exercises suggest that the step-face surface of the cellulose micro-fibril is most likely the preferred site of catalytic attack by the hydronium ion in thermochemical hydrolysis.

C Milestone #406, Complete Installation of Pilot Scale Pneumapress Filters (8/31/02)
The pilot scale Pneumapress filter fabrication and assembly is nearing completion and is expected to be delivered to NREL in early April. Numerous activities that will support installation and operation of the Pneumapress are underway, including horizontal pretreatment reactor improvements, pressurized Pneumapress feed vessel integration, PDU utilities tie-ins, ancillary equipment specification, and environmental safety and health (ES&H) reviews. Initially, operation of the filter will be validated under ambient temperature conditions (subject of a P milestone to be reported on 6/30/02), followed by operational readiness under elevated temperature conditions.

P Milestone #410, Expert Panel Identification (6/30/02)
This milestone will identify experts who are willing to participate in the development of criteria and the selection and ranking of pretreatment technologies that can potentially meet advanced technology process performance and cost targets. These same experts will serve on the review panel for the Advanced Pretreatment Project Gate B review meeting scheduled for November, 2002. One issue that requires discussion before proceeding with the identification of expert panel members is the role of technology providers and researchers who are pursuing a particular pretreatment approach in the evaluation and selection process.

P Milestone #412, Installation of Pilot Scale Pneumapress Filter for Ambient Temperature Operation (6/30/02)
The pilot scale Pneumapress filter fabrication and assembly is nearing completion and is expected to be delivered to NREL in early April. Initial operation will be validated

under ambient temperature conditions using a pretreated biomass slurry to verify solid-liquid separation and washing capability.

P Milestone #411, Lignin Properties from Hot Wash Process on Corn Stover (9/30/02) Researchers have characterized initial soluble lignin samples from corn stover generated in the bench scale hot washing apparatus. Additional samples from the pilot scale Sunds pretreatment reactor, the horizontal pretreatment reactor, and the Pneumapress filter will be generated and characterized. Representative samples will also be supplied to previously identified companies who have expressed interest in evaluating the potential value of low molecular weight lignins from the hot wash process.

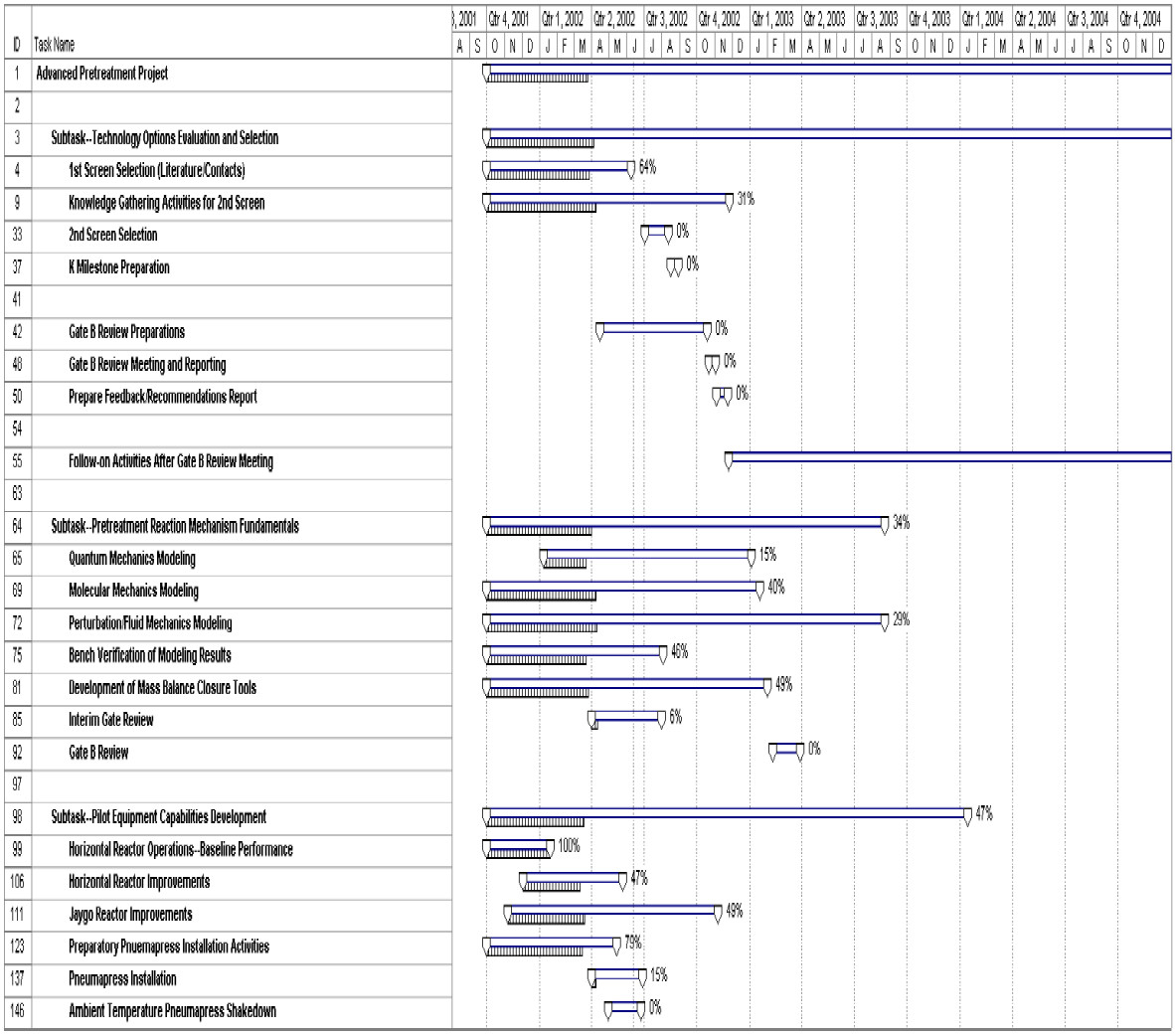

Figure 5. Advanced Pretreatment
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Highlights in the Technology Evaluation and Selection Subtask include on-going efforts with the Biomass Refining CAFI to coordinate several aspects of on-going CAFI projects. These efforts include common feedstock supply, storage, and compositional analysis, cellulase enzyme supply, storage, and characterization, providing analytical

chemistry procedures and student training on such procedures, and process engineering model development. While data gathering efforts for the CAFI projects is underway, the pace at which the data are becoming available for use in the process economic modeling evaluations has been somewhat slow, although it is now beginning to pick up. Also, while the pretreatment technologies being developed by the CAFI members covers a wide range of pretreatment technologies, there are several additional pretreatment technologies of interest that are currently not being supported. These include nitric acid pretreatment, wet oxidation pretreatment, and carbonic acid pretreatment.

A FY2002 subcontract solicitation is being prepared that will allow data gathering activities for some of these selected pretreatments in a similar manner to those being investigated by the CAFI researchers. This subcontract should be initiated by June, 2002. In addition, a task of the broad Process Engineering Team subcontract that is investigating pretreatment equipment materials of construction issues, primarily for alkali, neutral, and acid pretreatments other than sulfuric acid, is being funded through and will be monitored by the Advanced Pretreatment Project. This subcontract work should begin in May, 2002. All of these efforts will supply information needed for the August, 2002 K milestone that will identify pretreatment technologies with the potential of meeting advanced technology process performance and economic targets. Identified technologies will then be further evaluated by an external expert panel in the Advanced Pretreatment Project Gate B review meeting, currently scheduled for November, 2002. A small number of pretreatment technologies that have the greatest potential of meeting the advanced technology targets will be selected for further development at this review meeting.

Significant progress has been made in two key areas of research in the Pretreatment Reaction Mechanism Fundamentals Subtask during this reporting period. The first is the continued molecular modeling efforts from Dr. John Brady's lab at Cornell University to study the free energy molecular fundamentals of aqueous hydrolysis/fractionation of biomass. His lab's modeling work suggests that the step-face surface of the cellulose micro-fibril is most likely the preferred site of catalytic attack by the hydronium ion as opposed to the parallel face. This suggestion has an impact on how hydrolysis "effectors" may be designed and used to reduce severity in the thermochemical hydrolysis/fractionation of biomass.

The second achievement is furthering the established team approach to study and propose new global heterogeneous kinetics of the dilute-acid mediated pure cellulose hydrolysis. The members include Par Pettersson, a Ph.D. candidate from Mid-Sweden University, Qian Xiang, a Ph.D. candidate from Auburn University, Dr. Y.Y. Lee of Auburn University, and Robert Torget and Richard Elander from NREL. This group will be developing and incorporating mass transfer resistances into cellulose hydrolysis kinetic models as well as developing a comprehensive global model to explain the different hydrolysis rates obtained using pure cellulose in three reactor configurations: batch, fixed volume percolation, and the shrinking bed percolation reactor. Experimental work using the shrinking bed reactor at both Auburn and NREL have led to the conclusion that glucose/lignin interactions may be the reason for low glucose yields in traditional dilute-acid hydrolysis of lignocellulose and that using the shrinking

bed reactor with native yellow poplar minimizes these destructive reactions. Further work is needed however, to pinpoint why native yellow poplar gives high yields in this novel reactor and other lignocellulosic feedstocks fail to give yields above 65%.

Highlights in the Pilot Scale Equipment Capabilities Development Subtask include the initial operation of the recently reconfigured 200 kg/day horizontal pretreatment reactor system in the PDU. A high-speed motor capable of reducing the residence time in the horizontal pretreatment reactor by a factor of 5 or more has been installed. Initial residence time determinations have indicated that an overall residence time in the entire hot section of the reactor system of under 2 minutes can be achieved. Several runs have recently been completed under these short residence time conditions. We are continuing to push toward high solids loadings (>30%) in the horizontal reactor system, but these efforts have yet to prove successful. While the screw conveyors in the system should allow for effective transport of solids at any solids loading (a potential limitation of the Sunds hydrolyzer), we have been unable to achieve target temperatures in the horizontal reactor without high steam usage, resulting in solids loadings of no higher than 25%-30%. A likely cause for this is the fact that the horizontal reactor shell is only insulated and not jacketed, so heat losses to the environment are quite high and significant amounts of steam condensation may occur on the walls of the reactor. We are attempting higher temperature injection of dilute acid to compensate for this effect. An eventual solution to this problem could involve modifying and using the jacketed vertical stage reactor shell to replace the existing the horizontal reactor shell, with steam addition to the jacket. This would reduce the amount of steam that needs to be directly injected into the reactor, allowing for higher solids loadings at the targeted temperatures.

Good progress is being made in acquiring and installing the pilot scale Pneumapress pressure belt filter, which is the other major effort underway in the Pilot Scale Equipment Capabilities Development Subtask. The fabrication of the filter is nearly complete and it is expected to be delivered to NREL in early April. Significant pre-installation activities in the PDU are underway, including integration with the horizontal pretreatment reactor and Jaygo vessel (Pneumapress feed tank). The Pneumapress filter will serve many of the general solid-liquid separation needs in the PDU and will provide better process data on solid-liquid separations for use in process engineering models. In addition, this filter is capable of performing the hot separation and washing operations at the pilot scale for the hot wash process, which can potentially be employed in conjunction with many different pretreatment processes. Work in this subtask will lead to a June, 2002 P milestone involving ambient temperature operational readiness for the Pneumapress filter, and a August, 2002 C milestone involving elevated temperature operational readiness for the Pneumapress filter.

The Hot Wash Process Applications Subtask is gathering additional process data and representative samples from the hot wash process concept. Effects of this process on xylose yields and cellulase enzyme loading requirements are being determined. Current work is being conducted in the bench scale Parr pretreatment reactor and percolation hot wash system. Once the pilot scale Pneumapress filter is operational, much of the work in this subtask to generate representative materials will be done at that scale. A key emphasis of this subtask is to fundamentally characterize the

properties of soluble lignin fractions resulting from the hot wash process and to provide representative samples of such fractions to interested industrial concerns in order to determine if any co-product opportunities are worth pursuing. This will be the subject of P milestone #411 to be reported in September, 2002.

Subcontractors

Y.Y. Lee, Auburn University
ADZ-1-31084-01
Investigation of Hemicellulose Hydrolysis Fundamental Kinetics Using Dilute Sulfuric Acid
7/30/01 - 4/30/02
Work on this subcontract during this reporting period produced batch xylan hydrolysis and xylose formation kinetic data for corn stover at several acid concentrations, temperatures, and residence times. Initial data in the flow through shrinking bed reactor at extremely low sulfuric acid concentration using corn stover was also reported. This subcontract will be extended with additional funds and tasks in May, 2002.

Tim Eggeman, Neoterics International
LCO-1-31055-01
Process Engineering Modeling Support
1/9/02-11/01/02
This subcontract has been developing ASPEN-based process engineering and economic models for use in the evaluation of various pretreatment technologies. In this reporting period, efforts have been focused on model development for the AFEX/FIBEX pretreatment process (Bruce Dale, Michigan State University) and the ammonia recycle percolation process (Y.Y. Lee, Auburn University). This subcontract was initially funded by Process Engineering Studies —contract extension to cover model for additional pretreatment technologies is being funded by the Advanced Pretreatment Project.

John Brady, Cornell University
XDH-0-30009-01
Molecular Modeling of Structured Water Boundary Layer Under Hydrolysis Conditions
9/15/01 – 7/20/02
The subcontractor has conducted simulations that theoretically verify the hypothesis that structured water exists at the surface of cellulose at the high temperatures of dilute acid hydrolysis and that a significant density barrier exists due to this structuring which would affect free diffusion of released products to the bulk medium. Differences in the arrangement of water molecules at the step face of cellulose microfibrils as compared to the parallel face seem to indicate significantly less structuring of water molecules and lower water density gradients at the step face. A manuscript has been prepared and submitted to *Biopolymers* entitled “Computer Simulations of Water Structuring Adjacent to (1,0,0) Microcrystalline Cellulose Iβ Surfaces,” by C.E. Skopec, P. Zuccato, J.W. Brady, R.W. Torget , and M.E. Himmel.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- R. Elander, R. Torget, K. Ibsen, and J. McMillan attended several meetings of the Biomass Refining CAFI in Reno, NV on November 6-9, 2001.

- R. Elander met with Thomas Hanley and Sarah Priddy of the University of Louisville to discuss progress on the computational fluid dynamics DOE subcontract in Reno, NV on November 6, 2001.

- R. Elander participated as a member of the Panel of Experts for a review of the Dartmouth College/University of Sherbrooke/Purdue University pretreatment fundamentals project in Hanover, NH on November 29, 2001.

- R. Elander and M. Tucker inspected progress on the fabrication and construction of the pilot scale Pneumapress filter and discussed final control system programming at Pneumapress Filter Corp. in Richmond, CA on March 11, 2001.

- R. Elander and M. Tucker participated in the final pressure containment test for the pilot scale Pneumapress filter at Pneumapress Filter Corp. in Richmond, CA on March 26-27, 2001.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- Elander, R., Nagle, N., and Torget, R. "Examining the Potential for Waste Product Reduction and Energy Saving Techniques in Thermochemical Dilute Sulfuric Acid Processes for Biobased Products and Bioethanol Production." Oral presentation at the American Institute of Chemical Engineers National Meeting, Reno, NV, November 6-9, 2001.

- Torget, R.W., "Use of Molecular Mechanics, Perturbation Mechanics, and Quantum Mechanics as Tools for Biomass Fractionation Reactor Designs." Oral presentation at the American Institute of Chemical Engineers National Meeting, Reno, NV, November 6-9, 2001.

- Dale, B. (Michigan State University), Elander, R., Holtzapple, M. (Texas A&M University), Ladisch, M. (Purdue University), Lee, Y. (Auburn University), Torget, R., and Wyman, C. (Dartmouth College). "A Consortium for Coordinated Development of Leading Biomass Pretreatment Technologies." Oral presentation at the American Institute of Chemical Engineers National Meeting, Reno, NV, November 6-9, 2001.

- Elander, R., Nagle, N., Tucker, M., Ruiz, R., Rohrback, B., and Torget, R. "Initial Results from a Novel Pilot Scale Pretreatment Reactor Using Very Dilute

Sulfuric Acid on a Hardwood Feedstock." Accepted for presentation at the 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, April 28-May 1, 2002.

Scientific Journals: Papers Accepted for Publication

- Nagle, N., Elander, R., Newman, M., Rohrback, B., Ruiz, R., and Torget, R. "Efficacy of a Hot Washing Process for Pretreated Yellow Poplar to Enhance Bioethanol Production." Accepted for publication in *Biotechnology Progress.*

Summary of Technical Achievements or Results

Enzyme Research

Cellulase technology research during this reporting period has focused on Cellulase Fundamentals, CBH I Expression, and Hemicellulase/Accessory Enzymes projects.

Work on the Cellulase Fundamentals project was conducted at NREL, Cornell University (Brady and Wilson), and the University of Arkansas (Sakon). In support of Cellulase Fundamentals, NREL researchers have designed a directed evolution campaign to improve the performance of an actinomycete beta-glucosidase at elevated temperatures. NREL is also developing insights into the mechanism of cellulase action using the diafiltration saccharification assay (DSA) and new closed system cellulase assays. Cornell University continues to work on the computer modeling of the water boundary layer recently discovered to reside above the planar and step faces of cellulose. By years end, work done by this subcontractor will clarify the potential benefit offered by thermal tolerant cellulases.

In the CBH I Expression project, NREL initiated work to express *cbh1* from Baculovirus. The Hemicellulase/Accessory Enzyme project has recently focused on a Bioinformatics approach for cataloging potentially useful new enzymes. Overall, this research is following several paths, including literature reviews, screening of fungal cultures for enzymatic activities, and new genetic approaches to finding novel enzymes.

Cellulase Subcontract Liaison has focused on the benchmark testing of "state of the art" commercial cellulase samples from Genencor International and Novozymes and organizing subcontractor research update meetings.

General Technical or Scientific Progress

Hemicellulase and Accessory Enzyme Project

This project is primarily designed to determine if biomass conversion to free sugars can be carried out more efficiently with less severe pretreatment and a greater variety of enzymes. The main goals are determining biomass digestibility after alternative pretreatments (non-dilute acid) and characterizing non-cellulase enzymes that can

enhance this conversion. The research is following several paths, including literature reviews, screening of fungal cultures for enzymatic activities, and new genetic approaches to finding novel enzymes. The Hemicellulase/Accessory Enzyme Project is a new project for FY2002 and has undergone significant changes since its inception, mainly in advancing the timetable and scope of molecular screening for new enzymes.

Milestone Progress/Completion

P Milestone #392, Review of Plant Cell Wall Synthesis and Structure (3/31/02)
This milestone is essentially a literature review, and is progressing however the due date has been changed to mid-May. The review will cover biomass cell wall composition with emphasis on corn stover, the enzymology required for deconstruction of biomass, and the applicability of enzyme uses in conjunction with alternative pretreatments.

P Milestone #393, Opportunities for the Use of Hemicellulases and Accessory Enzymes in Conjunction with Advanced Biomass Pretreatments (6/30/02)
This work entails screening for activities necessary for enhanced conversion of alternatively pretreated biomass and determining economically viable scenarios for using such enzymes. These processes may involve enzymatic treatment of biomass before, during, or after pretreatment. A meeting with a process engineering task subcontractor (Neoterics, International) has been held to discuss the economic modeling of combining hemicellulases and accessory enzymes with some of the advanced pretreatments. Neoterics, International is currently looking at several scenarios, including enzyme addition to low temperature, high pH pretreatment and immediately following ammonium fiber explosion pretreatment. This work is on schedule.

P-Milestone #391, Hemicellulase/Accessory Enzyme Interim Stage A Review (7/31/02).
This review will cover the literature review and initial economic modeling work as well as the preliminary characterization of in-house fungal activities and molecular screening data. We will also seek input on future directions regarding pretreatment/enzyme integration and screening and improvement of novel enzymes.

Progress Highlights and Issues

This project is currently focused on three main areas; a literature review of plant cell wall composition and the effect of advanced pretreatments on biomass, development of new *in silico* screening techniques for elucidation of novel potential enzymes, and screening of white-rot fungi for hemicellulase activities.

The literature review will be available as a P-milestone report in mid-May and will include sections on corn stover composition, enzymatic deconstruction of biomass, and the potential uses of enzymology in conjunction with advanced pretreatment technologies.

The molecular genetics work is focusing on grouping the known glycosyl hydrolase enzymes into groups that share certain sequence homology. Conserved regions in these families will then be used to design molecular tools to extract new similar genes from both cultured organisms and from uncharacterized gene sequences in public databases. Several families have already been broken down into subfamilies containing useful sequence motifs. Work is continuing on other glycosyl hydrolase families.

The following fungi have been grown and screened under solid-state fermentation conditions on dilute acid pretreated corn stover; *Ceriporiopsis subvermispora, Cyathus stercoreus, Dichomitus squalens, Lentinula edodes, Penicillium pinophilum, Phanerochaete chrysosporium* Sc-26, *Phanerochaete chrysosporium (*BKM), *Trametes cingulata (*University of Minnesota*), Trametes cingulata (*ATCC), and *Penicillium funiculosum.* We are assessing the activities present in each culture and are also assessing GCI and Novozymes benchmark cellulase preparations for hemicellulolytic activities. The activities present in each of these strains will be quantified kinetically and correlated to protein content.

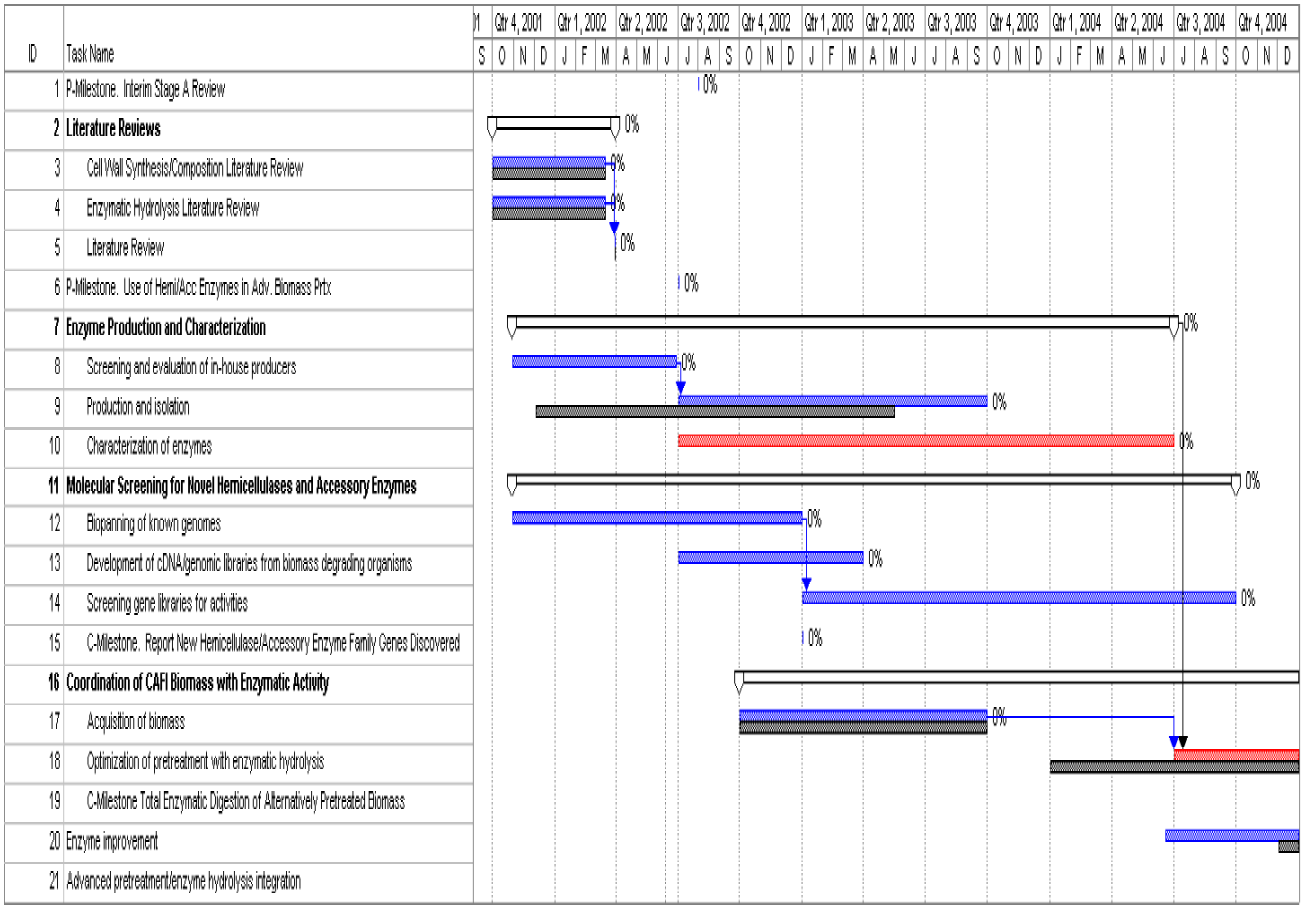

Figure 6. Hemicellulase and Accessory Enzyme Project
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Subcontractors

Simo Sarkanen, University of Minnesota
XCO-1-31048-01
Lignin Depolymerase from *Trametes cingulata*
This subcontract encompasses several goals including:

- Grow and induce *Trametes cingulata* for the production of lignin depolymerase (LD) and provide broth to NREL.
- Purify quantities of LD and transfer to NREL for amino acid sequencing
- Design genetic probes based on the NREL sequence data.
- Develop cDNA library and screen for LD
- Clone and express LD in *E. coli*
- Transfer LD assay(s) to NREL for confirmation and validation
- Continue to improve LD assay and purification protocol

Dr. Sarkanen at the University of Minnesota has been continuing production of lignin depolymerase by Trametes cingulata and furnishing the crude culture broth to NREL. He is also working on purifying the lignin-depolymerizing enzyme utilizing a native flatbed isoelectric (IEF) focusing technique. The current effort has been focused towards purifying enough enzyme to obtain sequence data in order to enable the cloning and expression of the gene. Recent efforts in this area have been tedious at best; as the enzymes are produced in extremely low levels and purification by standard protein methods are not applicable due to the presence of lignin.

General Technical or Scientific Progress

Cellulase Fundamentals

We are studying three well-defined aspects of cellulase biochemistry that pertain to improving cellulase performance and transferring new insights to the appropriate subcontractors and/or the cellulase research community through publications and presentations. These three aspects are endoglucanase, exoglucanase, and cellulase/cellulose biochemistry and are outlined by the milestones below.

Milestone Progress

C Milestone #384, Cellulase Fundamentals Directed Evolution Campaign of Endoglucanase or beta-Glucosidase (9/30/02)
Our target is to increase the thermal tolerance of a key beta-glucosidase. The rationale for this objective is that cellobiohydrolases are very sensitive to cellobiose and considerable research is underway in the program to increase the saccharification temperature. We have chosen the beta-glucosidase from *Thermobifida fusca (*a soil actinomycete) as the candidate enzyme for our directed evolution work. *T. fusca* utilizes cellulose in nature and produces a cell-associated beta-glucosidase, as well as many other cellulases. The *T. fusca* genome is now available and two sugar permeases (*bgl*A, *bgl*B) and a beta-glucosidase (*bgl*C) have been found in the *Bgl* operon. The *Bgl*C protein has a temperature optimum of 50°C, a MW of 53.4 kDa, and 4 cysteines. *BglC* is expressed well from *E. coli* using the pET-26b vector, but cannot

perform at elevated temperatures. We received the gene for *BglC* from Cornell (D. Wilson) in December, 2001 through a material transfer agreement (MTA) with Cornell University. Error-prone PCR mutagenesis of *bgl*C using a Clontech Diversify PCR Random Mutagenesis system has been completed and modified DNA is now ready to transform *E. coli* for mutant library construction.

P Milestone #386 Conduct Directed Evolution Experiment on Endoglucanase or beta-Glucosidase (5/30/2002)
We plan to conduct directed evolution (DE) campaign(s) employing at least 100,000 clones in the search for increased thermal tolerance or activity range of enzyme. A novel high-throughput screen using the Autogen robotics colony picker has been developed and validated for this milestone. A beta-glucosidase insoluble substrate, X-glc, will be used to signal positive clones from an 80^{o}C temperature challenge after spotting the error prone PCR library on nitrocellulose membranes.

P Milestone #387, Publish Role That Boundary Water Plays in Cellulase Action (9/30/2002)
We will provide a thermodynamic description of the water boundary layer and describe its possible role in cellulase action. Two manuscripts describing the results of the molecular mechanics (MM) modeling of cellulose/water structure are on track and will be submitted to *Biopolymers* as planned.

P Milestone #388, Reconcile Differences Observed for DSA and SSF Cellulase Performance (9/30/2002)
We intend to provide performance-based and/or kinetic descriptions of mechanisms involved in dialysis and SSF cellulase action that explain observed differences, and recommendations regarding appropriate use and interpretation of the assays. DSA and closed system cellulase assays are being performed with Spezyme and Celluclast commercial preparations. The milestone is on track and should be completed as planned.

Progress Highlights and Issues

A manuscript recently submitted to *Protein Engineering* reports two sets of simulations: standard molecular dynamics (MD) simulations of the previously obtained endoglucanse-substrate complex in aqueous solution, and a search for alternate conformations of the loop containing E2Asp79 using constrained MD simulations and energy minimization calculations. In the latter calculations, alternate conformations were examined to look for any which had mechanical energies comparable to or lower than the energy of the crystal structure. In particular, because mutagenesis data demonstrated the critical role of Asp79 in the catalytic activity, alternate conformations for the loop containing residue 79 were sought which placed this residue closer to the scissile bond of the substrate. The MD simulations were used to refine the energy-minimized structures, exploring the configuration space around these structures to determine if there were other nearby conformations separated by energy barriers, and also to give a picture of the motions of the enzyme-substrate complex that might contribute to the catalytic mechanism.

Understanding the interactions between cellulases and cellulosic substrates is critical to developing an efficient artificial cellulase system for converting biomass to sugars. Molecular mechanics simulations at Cornell University have been used to model the structure of water adjacent to microcrystalline cellulose Iβ step and planar faces at two different temperatures: 300K and 478K. Two manuscripts describing this work are in preparation and will be submitted to *Biopolymers* for review. We expect these to be landmark papers in the field.

Improved methods for high throughput screening (HTS) of clones obtained from directed evolution studies of beta-glucosidases and endoglucanases are also being developed at NREL. The required screening for directed evolution of cellulases is extensive; hundreds of thousands to millions of clones need to be screened for the desired traits. HTS at NREL is also focused on the problems of assaying cellulase preparations by automated filter paper type protocols.

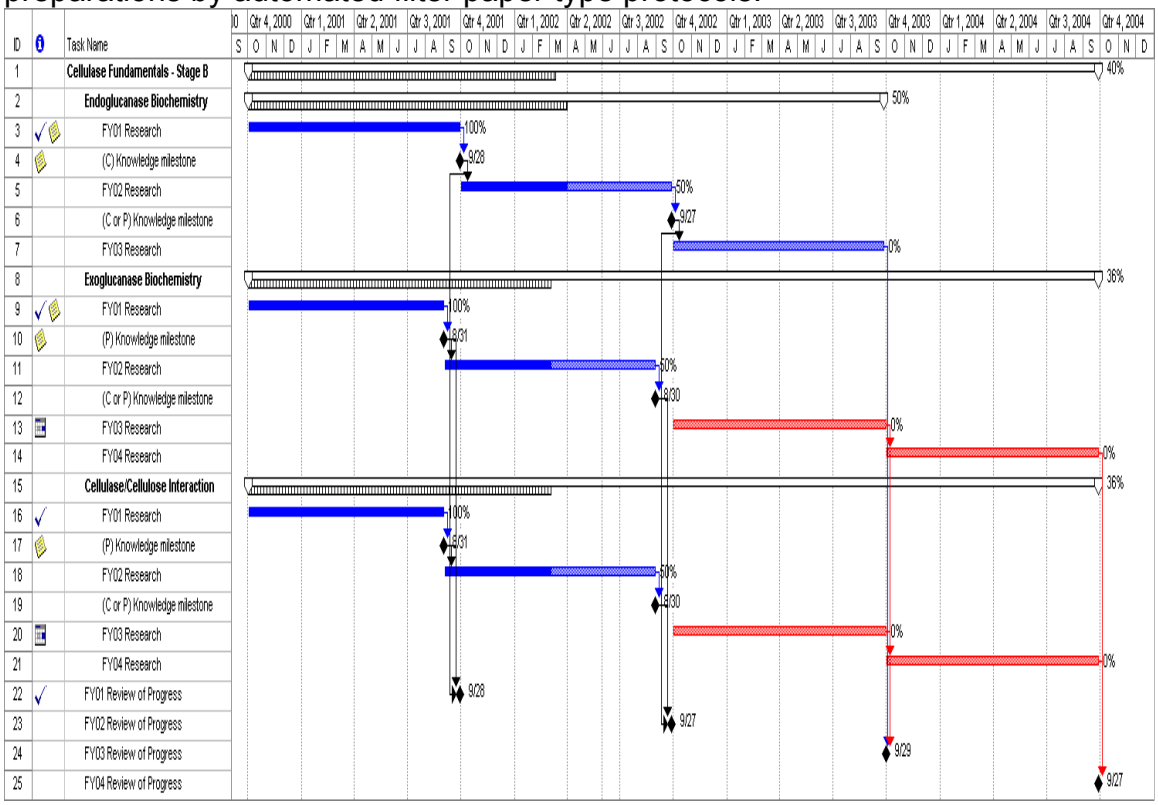

Figure 7. Cellulase Fundamentals Project
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Subcontractors

Cornell University, David Wilson
XDH-9-29048-01 mod 4
"Improving *Thermomonospora fusca* Cellulase Enzymes by Protein Engineering"
6/17/99-11/16/01

The subcontractor studied the construction of a set of E2 mutants in which selected residues were changed to Pro to try to increase thermostability. The residues were selected by looking for residues that were Pro in a related protein, but not in E2. The mutants were then screened in silico with energy calculations to rule out mutations that created strain. Six single mutants were constructed. Unfortunately, none of the mutants had higher thermostability. However, overall this set showed less decreased thermostability than the first set of mutants which we had made where we changed residues without checking that Pro was present in a related enzyme. Four of the mutants retained at least 80% of the activity of the wild type on different substrates. The subcontractor also studied the effect of temperature on the activity of E2 and on synergistic mixtures containing E2 using several substrates. An interesting finding is that there is a deviation from linearity in the Arrhenius plots for E2 on crystalline substrates but not on soluble ones. This deviation is caused by activity increasing at a faster rate then expected between 30 and 50°C. The rate of filter paper digestion by E2 increased 955% (rather than 100% as seen with the soluble substrate CMC) when the temperature increased from 30 to 40°C and another 224% from 40 to 50°C. In contrast, the filter paper digestion rate for E3, an exocellulase, increased only 53% from 30°C to 40°C and then jumped to 675% from 40°C to 50°C. The rate for the synergistic mixture of E2 and E3 increased 361% from 30°C to 40°C and 188% from 40°C to 50°C. At this time, they are trying to see experimentally if this is due to a change in cellulose hydration or structure or a change in the enzyme.

Cornell University, John Brady
ADH-1-31071-01"Molecular Modeling of the Interaction of Cellulose with Cellulases and Catalysts"
09/20/01-09/19/02
Mutagenesis experiments suggest that Asp 79 in cellulase Cel6A (E2) from *Thermobifida fusca* has a catalytic role, in spite of the fact that this residue is more than 13 Å from the scissile bond in models of the enzyme-substrate complex built upon the crystal structure of the protein. This suggests that there is a substantial conformational shift in the protein upon substrate binding. Molecular Mechanics simulations were used to investigate possible alternate conformations of the protein bound to a tetrasaccharide substrate, primarily involving shifts of the loop containing Asp 79. Several alternate conformations of reasonable energy have been identified, including one in which the overall energy of the enzyme-substrate complex in solution is lower than that of the conformation in the crystal structure. This conformation was found to be stable in molecular dynamics simulations with a cellotetraose substrate and water. Based on the results of MD simulations of the enzyme-substrate complex, a new catalytic mechanism is proposed. Using this mechanism, predictions about the effects of changes in Arg 78 were made which were confirmed by site-directed mutagenesis.

University of Arkansas, J. Sakon
XDH-0-30009-02 University of Arkansas
"Provide Support for Cellulase Engineering"
04/04/00-11/29/02
The goals of this subcontract include structure determinations of enzymes vital to the project objective. These data are also key for planning protein-engineering efforts.

The subcontractor has obtained crystals of glycosyl hydrolase Dex1 that diffract to about 1.7Å resolution using the University of Arkansas in-house X-ray source. The plate-like crystals diffract anisotropically. All bio-macromolecular crystals suffer from time-dependent radiation-induced loss of diffracted intensity to some degree. In many of these cases the radiation damage at room temperature makes it impossible to collect a complete data set from a single crystal. This problem can often be overcome by flash-cooling the crystal to very low temperature. The subcontractor has developed a method to flash freeze the Dex1 crystals. Flash freeze crystals are stored in vials for synchrotron trips scheduled for March 2002. Work has recently begun on a new cellulase isolated from *A. cellulolyticus* and diffractable crystals have already been obtained.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

M. Himmel agreed to serve as Chair of the Gordon Research Conference on "Cellulases and Cellulosomes" to be held in Proctor, NH July, 2003.

General presentations at NREL to Genencor, Novozymes, and numerous academic and congressional tour groups.

S. Decker gave numerous presentations of the HTS facility to academic, DOE, and USDA visitors.

S. Decker attended a workshop on Laboratory Automation in January, 2002.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

"Utility of Cellobiose Dehydrogenase in the Automated Measurement of Cellulolytic Activity, Stephen R. Decker, Edward W. Jennings, Todd B. Vinzant, William S. Adney, and Michael E. Himmel, The 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.

"New Glycosyl Hydrolases from *Acidothermus cellulolyticus*, Shi-You Ding, William S. Adney, Stephen R. Decker, John O. Baker, Ed Jennings, Todd B. Vinzant, and Michael E. Himmel, The 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.

"Proteases and the Extracellular Lignin Depolymerase Activity from *Trametes cingulata*, Yi-ru Chen, Todd B. Vinzant, Stephen R. Decker, Ed Jennings, Michael E. Himmel, and Simo Sarkanen, The 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.

"Changes in Cellulose Morphology of Pretreated Yellow Poplar During Enzymatic Hydrolysis," Mark Davis, John Baker Tauna Rignall, and Michael Himmel, The 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN May, 2002.

Scientific Journals: Papers Submitted for Review

"Outlook for Bioethanol Production from Lignocellulosic Feedstocks: Technology Hurdles," J.S. Sheehan and M.E. Himmel, AGRO-food-INDUSTRY HI-TECH, TeknoScienze, Milano, Italy, 2001, In Press.

"Catalytically Enhanced Endocellulase Cel5A from *Acidothermus cellulolyticus*," J.O. Baker, J.R. McCarley, R. Lovett, C.-H. Yu, W.S. Adney, T.R. Rignall, T.B. Vinzant, S.R. Decker, J. Sakon, and M.E. Himmel, J. Biotechnology, 2002, Submitted.
"Computational and Experimental Studies of the Catalytic Mechanism of *Thermobifida fusca* cellulase Cel6A (E2)," P. Kanchanawong, G. Andre, R. Palma, H. Cho, X. Deng, D. Irwin, M.E. Himmel, D.B. Wilson, and J.W. Brady, Protein Engineering, 2002, Submitted.

Summary of Technical Achievements or Results

CBH I Expression

A Gate B review per the stage gate criteria was held on the CBH I expression project by a technical and management team on October 31, 2001. This project is a key research effort in support of the enzyme subcontracts with Genencor and Novozymes. To date, *T. reesei* CBH I is the most effective cellulase component known. The performance of this enzyme would be enhanced, however, if its use at elevated temperatures were possible. Protein engineering principles cannot be applied to the improvement of this enzyme unless effective hosts for the *cbh1* gene are found. Therefore, the development an advanced heterologous expression system for cellobiohydrolases that enables the production of functional protein for structural and biochemical studies is critical to the ultimate goal of improving enzyme function. Eukaryotic genes are generally difficult to express in heterologous hosts due to the extent of post-translational modification that occurs in both the native and the heterologous host. Post-translational modifications of proteins are responsible for the proper conformation, stability, and ultimately the function of enzymes secreted by most fungi. The types and extent of these modifications is even more pronounced for highly secreted proteins such as CBH I from *Trichoderma reesei*.

General Technical or Scientific Progress

Milestone Progress

P Milestone #380, Transfect Insect Cells Using Recombinant Baculovirus Encoding for Native CBH I (2/28/02)
The objective of this milestone was the successful construction of recombinant baculovirus and transfection of suitable insect cells with the *cbh*1 gene from *T. reesei*. This milestone was completed on schedule. In this milestone the following results were reported:

- The successful construction of the vectors necessary for producing recombinant baculovirus particles containing the *cbh1* gene from *T. reesei.*

- The successful transfection of insect cells using recombinant bacmids containing the *cbh1* gene, and the production of recombinant baculovirus.

- The successful production and secretion of functional rCBH I from pilot expression studies using the insect cell line *Sf*21. Western blot analysis of culture supernatants indicates that the rCBH I is secreted, is produced in suitable quantities, and shows no evidence of proteolysis.

C Milestone #381, Purify CBH I Expressed in Insect Cells (Go-No-Go Decision) (6/30/02)
The objective of this milestone is the production and purification of 1-10 mg of purified CBH I expressed from insect cells. The goal is to produce purified, active, functional CBH I from insect cells and to evaluate its biochemical properties. We have already demonstrated the production of active full length CBH I expressed in insect cells and have begun to produce larger quantities of the enzyme to analyze. This milestone contains a decision point which will be made after careful evaluation of the rCBH I protein produced from insect cells. If this system is deemed suitable for engineering cellobiohydrolases, we will begin to engineer modified cellobiohydrolase genes and evaluate specific targets for improved performance characteristics, such as improvements in the thermal tolerance of the protein.

C Milestone #382, Report Comparison of Aspergillus Species Produced CBH I Full Length Enzyme" (9/30/02)
The objective of this milestone is the cloning and purification of 1-5 mg of purified CBH I expressed from *A. awamori, A. niger*, and *A. nidulans* and to determine the biochemical and kinetic characteristics of each of these enzymes. The applicability of each respective host system will be evaluated for their application of enzyme engineering strategies including site-directed mutagenisis, directed evolution and chimeragenesis.

Progress Highlights and Issues

Cellobiohydrolases, including CBH I from the mesophilic mold *Trichoderma reesei*, are major contributors to hydrolysis of microcrystalline cellulose. In order to enzymatically hydrolyze biomass more effectively to its component sugars, it is essential that key improved enzymes are either developed or discovered. This year we are focusing on hosts that can provide many of the post-translational modifications that are required for the activity of eukaryotic proteins such as glycosylation, subunit cleavage, disulfide bond formation, and proper folding of proteins. Baculovirus expression systems for heterologous gene expression have been widely and successfully implemented in the production of correctly folded and functional foreign proteins including many examples of fungal genes. Recombinant proteins that are expressed in this host are generally correctly processed, modified, and are functionally active. We have been successful in producing full-length rCBH I in insect cells and are currently evaluating its biochemical properties. Testing of insect cell culture supernatants also indicate that there are no other interfering enzyme activities, thus allowing the use of sensitive assays for the detection of improved activities.

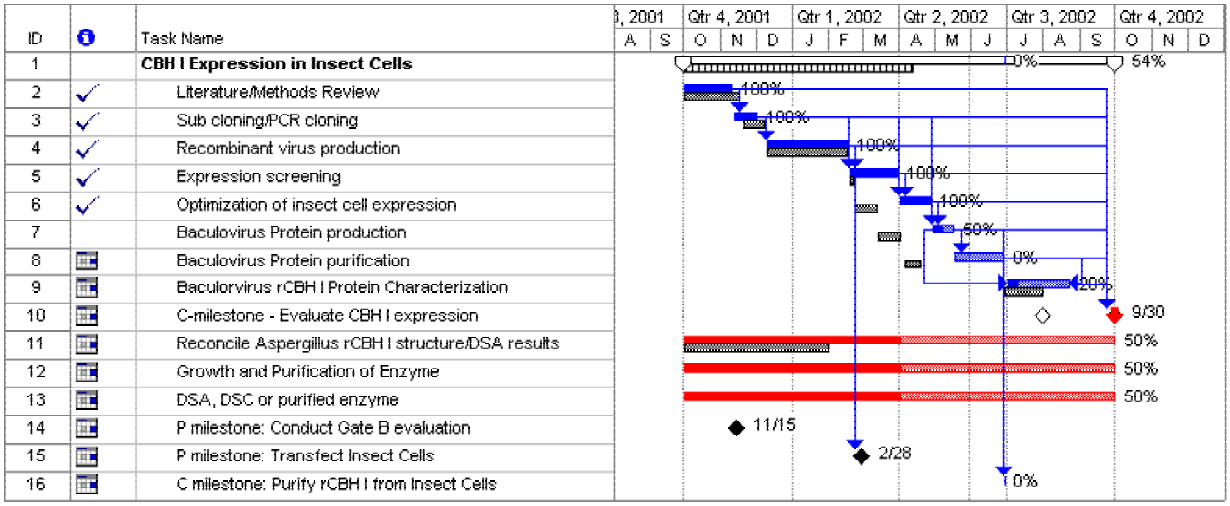

Figure 8. CBH I Expression Project
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Presentations on CBH I expression were made by Bill Adney to Genencor and Novozymes during subcontract review meetings at NREL. Their suggestions and feedback have been incorporated in the project plans.

Summary of Technical Achievements or Results

CRA/NCGA CRADA on Arabinose Yeast Transport

This is a funds-in CRADA project with Corn Refiners Association (CRA) and National Corn Growers Association (NCGA). The primary objective is to identify an efficient L-arabinose transporter for yeast. The lack of sufficient arabinose transport in *S. cerevisiae* has been determined to be one of the major hurdles that need to be overcome before efficient L-arabinose-fermenting yeast can be developed. Little is known about arabinose transport in *Saccharomyces* and almost nothing is known about it in other yeast species. Although *S. cerevisiae* have very poor arabinose transport activity, there are other species of yeast that grow well on L-arabinose and, hence, must have an efficient mechanism for its transport. Once we have identified one or more yeast species that have suitable arabinose transporter(s), we will attempt to discover and isolate the gene(s) involved and engineer it for expression in *S. cerevisiae*.

General Technical or Scientific Progress

Milestone Progress

C Milestone #426, Strain With Suitable L-arabinose Transport (9/30/02)
This milestone is expected to be completed by 9/30/02.

P Milestone #424, Yeast Strains from Various Species Capable of Growth on L-arabinose (2/28/02)
This milestone was completed on schedule. We obtained 144 strains from 115 different species of yeast that are expected to grow on L-arabinose. Detailed studies of their growth on L-arabinose and L-arabinose uptake measurements are in progress.

P Milestone #425, Selection of Strains for Kinetic Measurements of L-arabinose Transport (6/30/02)
This milestone is expected to be completed by 6/30/02.

Progress Highlights and Issues

Procurement of the yeast strains: Through extensive literature surveys and examination of descriptions of hundreds of different species of yeast in the catalogues from various culture collections, we identified a number of strains that may be suitable sources of an efficient transporter(s) for L-arabinose. We have obtained 144 of these strains: 25 from the American Type Culture Collection (ATCC) in Manassas, Virginia; 29 from the Northern Regional Research Laboratory (NRRL) of the USDA in Peoria, Illinois; and 90 from Centraalbureau voor Schimmelcultures (CBS) in Utrecht, Netherlands. These strains represent 115 different species of yeast.

Growth of the yeast strains: We revived the strains obtained from various sources and isolated purified colonies of them. Cultures of purified colonies were inoculated at low initial density yeast extract-peptone-arabinose media. The cultures were analyzed for growth at various time intervals by monitoring optical density. In addition, the culture supernatants were analyzed by HPLC to determine efficiency of L-arabinose utilization as well as for the presence of any unusual metabolites. We have analyzed 94 strains thus far. Twenty-six of the strains utilized all the L-arabinose in the medium within 24 hours and 30 other strains used up all the arabinose within 48 hours. Other strains used some or all of arabinose at lower rates. We assume that the strains that consume L-arabinose at faster rates would have an efficient transport system for the sugar. Although presence of efficient transport in slow arabinose-utilizing strains is not ruled out since they may have reduced growth rated due to other limitations, we are concentrating our further analysis on strains that consumed arabinose quickly.

L-arabinose transport: We set up the facilities and prepared appropriate SOPs for performing L-arabinose transport studies using ^{14}C-L-arabinose. We purchased the radioactive substrate from American Radiolabeled Compounds (ARC), Inc., which was the only supplier of this substrate. However, during our study we discovered that because of significant levels of impurities in the preparation supplied by ARC, the uptake measurements were not valid. ARC was not able to solve this problem. We

have are now doing transport studies with pure preparations of ^{3}H-L-arabinose. We also contracted Moravek Biochemical to custom-synthesize ^{14}C-L-arabinose. This compound does not have the impurities present in the ARC material. Transport experiments with ^{3}H-L-arabinose and ^{14}C-L-arabinose have indicated that both are essentially equivalent. We are doing most of our studies with ^{3}H-labeled substrate since it is more readily available.

The ideal source for an L-arabinose transporter would be a strain that consumes the sugar efficiently and uses a facilitated diffusion mechanism for the uptake of the sugar. An active transport mechanism requires energy for the uptake and such a requirement may reduce the overall efficiency of fermentation. We will complete the evaluation of growth on L-arabinose of all the strains. We will continue to determine L-arabinose transport in the strains that utilize the sugar efficiently. We hope that some of these strains use facilitated diffusion for L-arabinose transport, as is the case for glucose transport in *S.* cerevisiae. Otherwise, we will choose a strain that consumes L-arabinose efficiently and has a high rate of L-arabinose transport.

Scientific Publications, Presentations And Other Activities

General Presentations/Travel

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

The following abstracts were accepted for poster presentation at the 24th Symposium on Biotechnology for Fuels and Chemicals, Gatlinburg, TN, April 28-May 1, 2002.

- "Construction of Xylose Utilizing *Zymomonas mobilis* Integrants Based on Strain ATCC31821". Yat-Chen Chou, William Howe, Kent Evans, Min Zhang

- "Development of Automated Enzyme Assays for Analysis of Pentose Utilization Enzymes in Cellular Extracts". Christina Eddy, Steven Decker, Qiang Gao, Dhinakar S. Kompala, James D. McMillan, and Min Zhang

ADVANCED CAPAPBILITIES AND SUPPORT

Summary of Technical Achievements or Results

Rapid Analysis (including ASTM)

Development of a new preliminary NIR/PLS method for the compositional analysis of corn stover liquid and slurry process intermediates is underway.

Instrument Selection: Information has been collected on instruments for NIR, Fourier transform infrared (FTIR) and Raman spectroscopy from Ocean Optics, Bruker, Yamato Scientific, Foss North America, Nicolet, Analytical Spectral Devices, Perkin Elmer, Applied Spectral Systems, Jasco, and Perten. This information includes instruments for solids analysis and field analysis as well as for the analysis of liquids and slurries. The Foss Multi-mode NIR spectrometer was selected for purchase because it offered controlled temperature modules and flow-through cells that might allow PLS method to move closer to the process line, minimizing sample preparation. This module included both reflectance and transmittance detectors and was available as an accessory for our existing spectrometers. The Foss Multi Module was ordered in November, 2001 and was installed early in January, 2002. Planned method development includes learning how to collect high quality spectra at elevated temperatures. Challenges associated with the spectroscopy of slurries samples will be explored.

Software Selection: The temperature module and transmission detector require Vision® software. This software is integrated with all of the Foss process instruments, so PLS method development using Vision software moves us closer to on-line process monitoring. An on-site training course on the Vision software package was held during the week of March 25, 2002.

Calibration Samples: The Enzyme Sugar Platform and Advanced Pretreatment projects will provide calibrations samples and compositional data for PLS method calibration.

General Technical or Scientific Progress

Milestone Progress

P Milestone #375 for Stage /Gate Placement by the 28th of February, 2002 has been completed. A gate placement review was held on February 27th. The project and current tasks were defined as orange line stage B. Reviewers comments were incorporated and a revised work plan submitted.

C Milestone #374, Preliminary Rapid Analysis Method for the Chemical Characterization of Liquid and Slurry Samples (9/30/02)

The work on this milestone is slightly behind schedule and will require an aggressive schedule for on-time completion.

P Milestone #376, Report Documenting ASTM E48.05 Subcommittee FY 2002 Accomplishments and Plans FY 2003 (9/30/02)
This milestone is expected to be completed on time.

Progress Highlights and Issues

Rapid Analysis Support of Other Research Projects

NIR corn stover feedstock method expansion is a continuing effort on track supported by Enzyme Sugars Platform and Advanced Pretreatment Projects. The method has been expanded to include samples from five new locations and samples representing 13 known varieties of commercially grown corn. Corn stover samples representing different anatomical tissues were also included in this method expansion. The improved stover feedstock equation, stover5b.eqa, was calibrated using new wet chemical methods that have been optimized specifically for the analysis of corn stover. All NREL LAPs are being revised to document these improvements. For the new calibration set, all new samples were analyzed according to the improved wet chemical methods. Where possible, additional wet chemical analyses were performed on the old samples and compositional data recalculated. The improvements made to the wet chemical methods allow better correlation with the NIR spectra and have expanded the PLS predictions to include additional biomass components. The updates were completed in January 2002. The outlier identification flags associated with the stover5b equation have been used to identify samples with unusual cell wall components including glucan, xylan, lignin, protein, and structural inorganic contents. Extreme samples are being analyzed by wet chemical methods and will be used to further expand the current calibration range. The new methods were used to screen approximately 3,000 Colorado grown corn stover samples for interesting compositional mutations.

NIR corn stover intermediates preliminary method expansion is continuing and is supported by the Enzyme Sugars Platform and Advanced Pretreatment Projects. The calibration set for corn stover process intermediates was expanded in December 2001 and now includes more than 100 samples from pretreatment experiments using different reactors at varying severities. The latest method update contains samples generated from the new 2001 harvest feedstock. Wet chemical compositional analysis was performed on each of the calibration samples using standard wet chemical techniques to determine the levels of lignin, glucan, xylan, arabinan, galactan, mannan, protein, extractives, and ash. A PLS equation was obtained that predicted compositional chemistry from the NIR spectrum with errors that closely matched those observed for the wet chemistry used in the method calibration. The method is designed for grab-sample analysis and requires only simple sample preparation. The new NIR/PLS rapid analysis method was successfully validated with a set of 20 independent samples with chemical compositions reflecting the entire calibration range. The NIR/PLS method, stovint4.eqa, is now being used to provide a quick estimate of the composition of pretreated corn stover. These quick estimates are

being used to set enzyme loadings for SSF experiments. A few samples of SSF process residues for corn stover have been received and preliminary tests suggest that SSF residues are clearly distinguishable from pretreated material and may require a separate PLS method for rapid compositional analysis

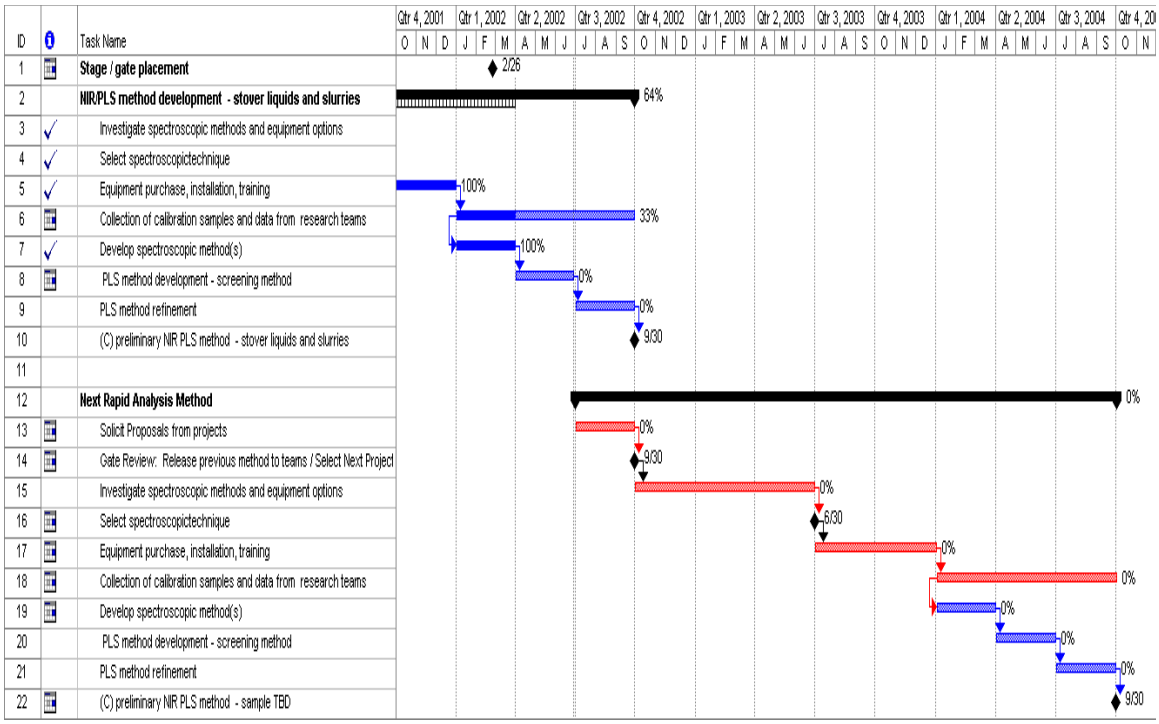

Figure 9. Rapid Analysis Project
Baseline plan (upper bar), current plan (lower diagonal bar), and progress (lower solid bar). Status date 3/31/02

ASTM

In December 2001, the American Society for Testing and Materials (ASTM) re-published seven methods for characterizing biomass through the E48 committee on Biotechnology and the E48.05 subcommittee on biomass conversion. These methods are updated and edited versions of standard methods originally published in 1995. The Biofuels Web site was updated to include links to the new ASTM standard methods.

Nine new NREL LAPs optimized for the compositional analysis of corn stover feedstocks and process intermediates have been prepared (see milestone #434 in Enzyme Sugar Platform Project). The LAPs are being written in ASTM compatible format and will be submitted for full E48 committee review and publication as standard methods. One representative from Cargill-Dow has expressed interest in joining the E48.05 subcommittee as a producer representing the bioenergy industry.

Scientific Publications, Presentations and Other Activities

General Presentations/Travel

- "NIR Rapid analysis Method for Corn Stover Process Intermediates" A. Sluiter, contributing authors presented at Chemistry of Bioenergy Systems Seminar, January, 2002.

- "Rapid Analysis: New methods supporting Ethanol Production from Biomass" B. Hames, A. Sluiter, R. Ruiz, C. Roth, S. Thomas, T. Hayward. Stage/Gate placement review, February 27, 2002.

- Bonnie Hames attended the fall meeting of the ASTM E48 Committee on Biotechnology in Rockville, MD in October 2001. Bonnie was elected as Vice-chair of the E48 committee on Biotechnology. She will continue to chair the E48.05 subcommittee on Biomass Conversion.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- "Quantifying fungal cell mass in the presence of lignocellulosics using near infrared spectroscopy and multivariate analysis," A. Tholudur, B. Hames, R. Meglen, T. K. Hayward, J.C. Saez, and J. D. Mcmillan, Poster to be presented at the 223rd national meeting of the American Chemical society in Orland FL, April 2002.

- "Rapid Biomass Analysis: New analytical methods supporting fuels and chemical production from Biomass" Bonnie R. Hames, Samie D. Sluiter, and Robert R. Meglen, Oral paper to be presented at the 223rd National Meeting of the American Chemical Society in Orlando, FL, April 2002.

- Rapid Biomass Analysis: New analytical methods supporting ethanol production from biomass." Bonnie Hames, Amie Sluiter, Tammy Hayward, Chris Roth, Ray Ruiz, David Templeton and Steve Thomas. Poster to be presented at AIChE 2002, Spring National Meeting, New Orleans, LA, March 2002.

- "Rapid Biomass Analysis: New tools for compositional analysis of corn stover feedstocks and process intermediates from ethanol production." Bonnie R. Hames*, Steve Thomas, Amie D. Sluiter, Chris J. Roth, David W. Templeton. To be presented at the 24th Biotechnology Symposium for Fuels and Chemicals, Gatlinburg TN, April 2002.

Peer-Reviewed Publications

- "Chemical Analysis of Wood Chips in Motion Using Thermal-emission Mid-Infrared Spectroscopy with PLS Regression", R. W. Jones,*, R.R. Meglen, B. R. Hames, and J. F. McClelland, Analytical Chemistry (2002), 74(2), 453-457.

- ASTM Standard Methods (republished):
  E1690-01 The determination of ethanol extractives in biomass
  E1721-01 The determination of acid-insoluble residue in biomass
  E1755-01 The determination of ash in biomass
  E1756-01 The determination of total solids in biomass
  E1757-01 The preparation of biomass for compositional analysis
  E1758-01 The determination of carbohydrates in biomass by HPLC
  E1821-01 The determination of carbohydrates in biomass by GC

Summary of Technical Achievements or Results

Process Engineering Studies

The Process Engineering and Analysis team completed several important studies in this reporting period, both as a part of specific research projects and also under the Process Engineering Studies project, which covers work in areas that do not easily fit into a single research project. These studies investigated crosscutting areas such as improving the stover to ethanol process design, carbon and energy balance modeling, and analysis methods development. The process engineering team strives to provide analysis expertise in both the technical and economic aspects of biomass research. Activities in this reporting period include:

- Publishing a process design report for corn stover to ethanol
- Placing two subcontracts to develop methods and tools for biorefinery analyses
- Improving our understanding and modeling of the energy balance for biomass conversion to ethanol
- Continuing phase 2 of the joint USDA/NREL project to identify synergies and cost reductions from co-location of starch and cellulosic ethanol plants
- Improving our analyses with cutting edge tools and methods including Monte Carlo risk analysis
- Providing the biomass-to-ethanol Aspen Plus model and support to several universities and consultants, both in the United States and internationally (India, Sweden)

In addition, the Process Engineering team supported the research efforts of almost all of the Biofuels projects; details are listed in the respective project sections.

- Developed a process design for the Enzyme Sugar Ethanol Platform project that provides research priorities and possible scenarios that, when coupled with research successes, create an economically compelling process design for stover-derived ethanol.

- Determined preliminary economics for co-location of a cellulosic ethanol plant with either an existing coal-fired power plant or a new (greenfield) dry mill ethanol plant.

- Conducted single-point sensitivity analyses on many of the process variables. Feedstock cost, enzyme cost, pretreatment and fermentation yields, and the cost of capital had the largest impact on the design economics.

- Completed a preliminary analysis of the impact of enzyme improvements on process economics for Novozymes, Inc. as a part of the Subcontract Liaison Project.

- Summarized the economic studies performed for the Sealaska project over the 4-year project to help in making the go/no go decision.

- Completed a review and update of the lignin upgrade process design and costs with an outside engineering firm, resulting in a design document. Reports from the Harris Group can be downloaded from the Biofuels Information Center's document database at http://www.ott.doe.gov/biofuels/info.html.

- Performed sensitivity analysis on pretreatment, saccharification, and fermentation parameters coupled with feedstock composition variations.

- Developed a model to perform technical and economic evaluations of base-catalyzed pretreatments for biomass with the Biomass Refining CAFI.

- Completed a pre-feasibility study of wheat straw to ethanol for the Swedish BioAlcohol Foundation through the Industrial Partnerships team.

- Developed a process model and refined economics for distillers grains conversion to ethanol in preparation for a Gate 2 review.

- Evaluated a new biomass fractionation process aimed at lignin removal.

- Performed analysis of processing options in support of the Broin/NREL CRADA.

General Technical or Scientific Progress

Milestone Progress/Completion

C Milestone #403, Joint publication with USDA on Process Economics Phase 2 Project (8/31/02)
This phase is aimed at finding cost savings through integration of starch and cellulosic ethanol technology. An updated simplified NREL model (matching the published stover design report) has been developed. This is an important first step in the work, because there have been significant changes to the process design since Phase 1

was initiated. This simplified model has proven useful outside of this project, for distribution to universities and other research institutions that can make use of the Aspen model to better direct their efforts. Preliminary co-location scenarios developed for the Enzyme Sugar Ethanol Platform project Gate 3 review showed that a high level of integration is necessary for economic improvement over a dry mill alone. The USDA is working on utilizing results from a dry mill survey, taken in 1998 by the USDA, to update the dry mill Aspen model.

P Milestone #402, Corn Stover Design Report (3/31/02)
Milestone was delayed until 5/31/02 and is expected to be completed on time.

P Milestone #401, Place Subcontract With Engineering Firm to Investigate Key Process Areas (3/31/02)
Milestone was delayed until 5/31/02 and is expected to be completed on time.

Progress Highlights and Issues

The corn stover process design report titled "Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis 2002 Update" is completed and going through peer review. This is the second design report the Biofuels program at NREL has created; the first was in 1999 for poplar feedstock. These reports, which are the culmination of process design studies with engineering firms, NREL research, and subcontract research, make information on the conceptual design, operating, and capital costs readily available to the ethanol community. The report will be available for downloading from the Biofuels Process Engineering Web site at http://www.ott.doe.gov/biofuels/process_engineering.html.

Applying to refinery optimization and process analysis techniques applied to a conceptual biomass refinery is the focus of two subcontracts currently underway, each aimed at providing pieces of an analysis method for determining the optimum design of a biorefinery. A strategy for developing biorefinery configurations, coupled with a programming tool to determine the benefits of different configurations, will provide much-needed path to understanding the opportunities and issues in a more objective way. A C milestone is planned in FY2003 to report on these advances.

Dynamically generated energy and carbon balances for the corn stover design case were created. The balances show where the energy or carbon is entering and exiting the process. The key advantages of these balances are that they show the process efficiencies of carbon and energy conversion to ethanol and byproducts such as electricity. Being able to dynamically generate the balances adds a new level of rigor in comparing and contrasting different process configurations and co-location options.

Subcontractors

Dartmouth, L. Lynd
ADZ-2-31086-01
Strategic Biorefinery Model
1/24/02 - 4/24/02

Analyze a biorefinery scenario from a strategic perspective -- develop scenarios for a slate of products and determine effect on the cost of ethanol production.
Subcontractor is in the initial information gathering stage.

Delta-T Corp, R. McNulty - Completed
ZCL-0-30008-01
Evaluation of the Potential for the Production of Lignocellulosic Based Ethanol at Existing Corn Ethanol Facilities
3/02/00 – 3/31/02
Feasibility study of dry mill co-location with gasification and syngas conversion.
Contract completed in this period; awaiting public version of report for publication.

Ingram-Howell, A. Van Draanen - Completed
ACL-1-30123-01
Hydrolysis and Fermentation of Pulp and Paper Wastes
10/9/00 – 3/15/02
A final report was issued to complete Task 5 activities, "Citric Acid Fermentation from Ingram-Howell Hydrolysate." The raw material was hydrolysate produced at the Ingram-Howell pilot facility in Decatur, TN. An *aspergillus niger* strain adapted to dextrose fermentation was used. The initial fermentation temperature was 35°C and then reduced to 30°C. Yields of 75 to 85% (calculated on hexoses) were achieved with fermentation times between 33 and 48 hours.

Ingram-Howell, A. Van Draanen - Completed
ADZ-1-31088-01
Permitting of Biomass Hydrolysis and Fermentation Facility
7/3/01 – 11/3/01
The subcontractor performed a comprehensive review of applicable local, regional, state, and federal regulatory issues & permitting requirements necessary to sit a Biomass Hydrolysis & Fermentation Facility at a site in Washington State. In addition, the subcontractor provided a checklist of key environmental issues that cover the mandatory submissions and questions/answers.

Harris Group, L. Montague - Completed
ACO-1-30131-01
Lignin Process Design Confirmation and Capital Cost Evaluation
3/30/01 – 3/31/02
Lignin to aromatic products process design review and capital costing.
Contract completed in this period with lignin process design review and costing completed; awaiting public versions of reports for publication.

Neoterics International, T. Eggeman
LCO-1-31055-01
Process Engineering Support for Biotechnology Center for Fuels and Chemicals
7/5/01 – 5/1/02
Supporting Process Engineering team in various Biofuels projects.
Working on the CAFI pretreatment modeling and economic evaluation. Two ammonia pretreatments modeled during this period.

Pacific Institute for Studies in Development, Environment and Security, G. Morris
XDH-0-30010-04
Logistics Support and Development of a Bioethanol Market in the Western US – Bioethanol/Biomass Power Co-location Study
8/15/00 – 4/30/02
Co-location studies for bioethanol facilities with biomass-fired or coal-fired power plants. Coal power plant co-location scenarios completed in this period. Final report in preparation.

University of Pittsburgh, J. Marano
ADZ-1-31093-01
Biorefinery Optimization Software
10/29/01 – 4/29/02
Software model development to optimize biorefinery process configuration.
Subcontractor is in the initial information gathering stage.

Purdue/Williams, M. Ladisch
ZCO-1-31023-01
Bridge to Corn Ethanol - Phase 2 Project
2/22/01 – 5/31/02
Pilot testing of hot water pretreatment on corn fiber.
Pilot equipment installed and experimental plan expanded in detail in this period. Pilot operation planned in the next reporting period.

Jerry Sinor Consulting, Inc., J. Sinor. - Completed
TXE-0-29113-01
Market Analysis for Lignin Products
1/12/00 - 1/11/02
Market analysis and information gathering for lignin upgrade project.
Telephone oil industry market survey completed in this period to understand the market for octane enhancers derived from lignin. Contract not renewed for third option year due to lignin project cancellation.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Ibsen, K., Eggeman, T. (subcontractor) Attended AIChE's annual meeting in Reno Nevada to meet with the Biomass Refining CAFI members, November 2001.

- Wallace, R. Attended the Greensboro, North Carolina Ethanol workshop entitled "Ethanol for Rural America", December 2001.

- Wallace, R. Attended the Governor's Ethanol workshop in North Carolina, December 2001.

- Aden, A., Wallace, R. Attended and presented at the follow-up “Fuel Ethanol Production in Colorado” workshop, Yuma, Colorado, December 2001.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

- Aden, A., Ibsen, K., Ruth, M. “Technical and Economic Feasibility Assessments in the DOE Biofuels Program“ poster was presented at the American Institute of Chemical Engineers Spring Meeting, New Orleans, LA, March 2002.

- Ibsen, K., Aden, A., Ruth, M., Jechura, J. “Improving the Near-Term Economic Viability of Lignocellulosic Ethanol” abstract was submitted for the 24th Symposium on Biotechnology for Fuels and Chemicals, April 28, 2002, Gatlinburg, TN.

- Jechura, J., Ibsen, K., McMillan, J.; "Encouraging the development of Biorefineries—development of qualitative and quantitative planning models" abstract was accepted for presentation at the 24th Symposium on Biotechnology for Fuels and Chemicals, April 28, 2002, Gatlinburg, TN.

- Atherton, B., Jechura, J., Ruth, M. “Overall Energy Balance for the Corn Stover to Ethanol Process” abstract was submitted for the 24th Symposium on Biotechnology for Fuels and Chemicals, April 28, 2002, Gatlinburg, TN.

- Jechura, J., "Encouraging the Development of Biorefineries" abstract was submitted for the 4th Agricultural Biotechnology International Conference, September 15, 2002, Saskatoon, SK, Canada.

General Technical or Scientific Progress

External Analytical

Milestone Progress/Completion

P Milestone #394, Place or Renew Service Contracts on Critical Laboratory Equipment and Instrumentation (03/31/02)
This milestone was completed on time.

P Milestone #395, Conduct QA/QC Performance Check of External Analytical Support Laboratories (06/30/02)
Work on this milestone began in April to allow for completion by the due date and is expected to be completed on time.

Progress Highlights and Issues

This task manages and coordinates external analytical chemistry support for the Ethanol Project. Two competitively awarded subcontracts for outside laboratory analytical support are currently active, one with Hauser Chemical Research and the

other with Industrial Laboratories. Initially placed in August 1999, both of these companies have since been validated by NREL to perform biomass compositional analysis based on standard NREL laboratory analytical procedures. When time or internal resources for analytical chemistry support are limited, samples are sent out to these external laboratories to expedite research progress. Periodic QA/QC checks are made by the NREL subcontract technical monitor to ensure that these laboratories continue to produce high quality analytical data.

Due to high sample loads expected during the 2nd half of this fiscal year, it will be necessary to delay, until FY2003, the re-competition process for external analytical support originally scheduled for this year. Site visits to the two laboratories will be scheduled during the month of March to discuss analytical issues, as well as our plan to extend the subcontracts one additional year.

Subcontractors

Hauser Chemical Research
JDH-9-18127-01
Compositional Analysis of Biomass Samples
08/05/99 - 08/04/02

Industrial Laboratories
JDH-9-18127-02
Compositional Analysis of Biomass Samples
8/12/99 - 8/11/02

The two laboratories analyzed a total of 19 solid biomass samples during this reporting period. Upcoming pretreatment experiments will be generating large sets of samples, a portion of which will be sent out for analysis.

Summary of Technical Achievements or Results

Industrial Partnerships

The final three months of the Cargill-Dow CRADA have been successfully completed. All four milestones in the third modification of the statement of work were completed on schedule. This CRADA project was an extension of a one-year award by DOE to Cargill-Dow, LLC to help the company prepare to use cellulosic material (such as corn stover) as feedstock instead of starch for its new biobased polylactic acid (PLA) plastic. Cargill-Dow is already producing and selling PLA under the trade name NatureWorks™. Success will allow Cargill-Dow to use lower cost and environmentally friendly feedstock, thereby allowing expansion of the market for PLA and possibly other chemical products.

The Industrial Partnership Team attended and were participants in four half-day colloquies held with DOE/OFD senior staff and senior representatives of leading firms in key industries (fuels, chemicals, food and fiber processing, biotechnology) to discuss the scope and scheduling of the planned DOE/NREL Enzyme Sugar-Ethanol

Platform Project. These meetings gathered valuable industrial input on key issues such as the required size of any future cost-share demonstration plant, possible biomass feedstocks, and required research to lower perceived risks for industrial investment in commercial-scale cellulosic biomass-to-ethanol plants.

Feedback from the these meetings is being used to develop and implement strategies for involving potential industrial partners in Enzyme Sugar-Ethanol Platform scale-up and commercialization. It is also being used to help design and direct the next phase of integrated development and testing of enzymatic conversion of cellulosic biomass to ethanol and high value products.

During this period, two subcontractors, Easterly Consulting and BBI, began work on detailed case studies of the potential technical and cost synergies of co-locating a bioethanol plant with an existing or planned coal-fired power plant. NREL staff and the subcontractors jointly developed detailed criteria to evaluate potential co-location options in the Midwest (BBI) and in the New England/New York State area (Easterly Consulting). During this period, the subcontractors put forward several attractive options for the location of a bioethanol plant next to or within a power plant, and data is now being collected on key issues such as feedstock availability and cost, and potential savings for the bioethanol plant from the use of excess power plant steam, power, and water treatment capacity.

Under the Collins-Pine subcontract, work on testing lignin residues as boiler fuel has been stopped due to the unsatisfactory performance of the subcontractor in creating the required boiler fuels, as well as reporting the detailed results of the creation of the lignin residues from the dilute acid two stage conversion of wood by-products to ethanol. NREL and DOE staff are working with the California Energy Commission (CEC) and the other participants to find a way to get the project back on track and to get a boiler test completed.

NREL subcontractor TSS Consultants has identified several opportunities for DOE and NREL to participate in pioneer projects that will demonstrate the cost-effectiveness of a new biomass to ethanol industry in California. The Industrial Partnerships team continues to search for ways to work with TSS and the CEC to make use of California's large forest and agricultural residue biomass resources and need for ethanol to promote commercialization of lignocellulosic biomass-to-ethanol conversion technologies in California. This effort has become particularly important with the planned phase-out in California of MTBE as a gasoline blending agent and the need for large quantities of ethanol to replace the MTBE.

General Technical or Scientific Progress

Milestone Progress/Completion

C Milestone # 396, Complete Evaluation of Bioethanol Co-Location with a Coal Power Plant in the Midwestern United States (8/31/02)
Two subcontractors were selected to conduct on-site research at three locations. Kick-off meetings were held with the sub-contractors to clarify issues of project scope,

deliverables, and integration of these efforts with ongoing NREL bioethanol cost modeling activities. One attractive co-location site in upstate New York has been identified, and the subcontractor has begun detailed analysis of feedstock issues and potential technical and cost synergies from co-location with the existing coal-fired power plant. Three Midwestern potential co-location opportunities have been selected from the initial ten proposed by the subcontractor. The two most favorable of these three opportunities will be used as case studies for potential technical and cost savings from co-location with an existing or planned coal-fired power plant. All tasks and deliverables are on schedule for the planned completion on August 31, 2002.

Subcontractors

Plumas Corporation, John Sheehan
XCO-0-29068-01
Plumas County Ethanol Project
2/17/00 - 9/31/02
This subcontract was placed for the purpose of closely integrating the work on this subcontract with the work proposed by the California Energy Commission's (CEC) project entitled the "Collins Pine Co-generation Project", managed by the Collins Pine Company of Portland, Oregon. The emphasis of this subcontract is to demonstrate the suitability of lignin residues from a 2-stage dilute acid ethanol process for use as a boiler fuel in the Collins-Pine boiler at Chester, CA. A review meeting on the overall progress of the CEC and NREL projects was held on December 20, 2001 in Sacramento, California. The review meeting was attended by representatives of the CEC, Collins-Pine, Plumas Corporation and NREL. The consensus decision by the attendees was that results-to-date were unsatisfactory.

Due to unsatisfactory progress on lignin generation and unsatisfactory deliverables, a stop work order was placed by NREL on January 17, 2002. A previous stop work order was placed by the CEC for the portion of the project that it was funding. In order for work under the NREL subcontract to continue in the future, the subcontractor must provide additional data as stipulated in the stop work order. Efforts continue between all parties involved continue to get satisfactory results and demonstrated progress. A final report will be issued once the additional data have been supplied and the co-firing tests can be completed.

N. Hinman, BC International (BCI)
ZCO-0-30019-01
Gridley Ethanol Project Development
11/30/00 – 6/30/02
A no-cost extension has been granted, extending the period of performance through 6/30/2002. Work has been completed under the following four tasks: 1) rice straw collection, baling, storage and transportation; 2) hydrolysis and fermentation research of rice straw; 3) lignin analysis and modeling research, bench-scale combustion; and 4) lignin handling, storage and ash handling and disposition. Two tasks, pilot-scale and large-scale lignin combustion tests, are being de-scoped from the original SOW, pending the approval by NREL of a letter request from BCI setting forth the reasons for their request. At this point, it looks like BCI has not been successful in securing the

needed project financing for the proposed Gridley project. We are waiting for process and engineering reports, a site commitment report, a project financing reports, and a final report.

Easterly Consulting, Jim Easterly
ACO-2-31092-02
Feasibility Study for Bioethanol Co-Location with a Coal Fired Power Plant in the Midwest
11/13/01 – 07/12/02
NREL had a kickoff meeting with subcontractor November 14, 2001. Currently, a site in New York has been chosen for the study, and subcontractor is researching local feedstock issues. The site offers attractive synergies for a possible co-location scenario including available low-cost steam, excess wastewater treatment capacity, available industrial building space with installed utilities, and a demonstrated willingness by the power plant operator to burn biomass. Future work will entail design and cost estimates, and financial evaluation of the site to be performed cooperatively between subcontractor and NREL. Other issues to be researched include environmental, socioeconomic, and market issues for bioethanol.

BBI, Mark Yancey
ACO-2-31092-01
Feasibility Study for Bioethanol Colocation with a Coal Fired Power Plant in the Midwest
12/4/01 – 7/3/02
NREL met with Mark Yancey and Mike Bryan of BBI for a kick-off meeting December 5, 2001. BBI will be researching two sites for the possible opportunity of co-locating a lignocellulosic-to-ethanol plant. From an initial list of ten Midwestern co-location options, BBI and NREL have narrowed the search to three sites located in Nebraska, South Dakota, and Indiana. Additional data collection, currently underway, will lead to a final selection of two sites for further analysis. Future work for each site will entail design and cost estimates, and financial evaluation of the site to be performed cooperatively between BBI and NREL. Other issues to be researched include environmental, socioeconomic, and market issues for bioethanol.

Loyd Forrest, TSS Consultants
YDH -0-30010-01
Logistics Support and Development of Biomass Energy in the Western United States
2/7/00 – 05/31/02
The decision of the governor of California to phase out MTBE by the end of 2002 has led to a great deal of private sector and governmental interest in in-state ethanol production. While the MTBE phaseout may be delayed (the decision is still pending), a number of the California agricultural counties are conducting reviews to determine if there is an economic case to be made for cellulosic ethanol plants, primarily using plentiful local agricultural residues or forest thinnings. The California Energy Commission is also exploring this option, with special emphasis on forest thinnings. TSS continued to provide reports and data to private sector stakeholders that are interested in creating a new commercial biomass-to-ethanol industry in the west. These stakeholders included county governments; farmer's groups and associations,

such as the Farm Bureau; winery and sugar beet growers associations; rice growers associations; owners of existing and proposed new biomass power plant associations; timberland and sawmill owners generating biomass wastes, and waste industry haulers; landfill managers; and transfer station operators. In addition, TSS worked with state and local government officials, including rural county supervisors, Resource Conservation Districts, utility and power plant regulators by providing information from DOE and NREL. These reports demonstrated the feasibility and key issues or barriers to creating a new biomass to ethanol industry that would use biomass wastes from forests, urban waste streams and agricultural residues.

TSS has identified several opportunity for DOE and NREL to jointly explore projects that will support the creation of a new biomass to ethanol industry in California. Co-location offers a potentially promising scenario in California as there are a number of operating biomass and coal-fired power plants in the state. There are opportunities to co-locate ethanol facilities as part of an existing or new proposed electrical power plant and the CEC has indicated an interest in exploring these options jointly with DOE and NREL.

M. Smith, MSS Consultants
YDH -0-30010-02
Logistics Support and Development of a Bioethanol Market in the Western U.S.
2/3/00 – 06/02/02
MSS Consultants served NREL as a liaison with state and local agencies, and private organizations with regard to biomass energy issues and reported its findings to NREL technical monitor in its monthly reports. Due to the government's phase out program of MTBE, North Eastern states as well as California are more open to ethanol as a fuel additive. An avenue open for possible advancement are specialty chemical and petroleum refining companies. Specialty chemical companies are interested in locating new sources for biomass-based pentose sugars, while refiners interested in assuring supplies of ethanol for future gasoline blending.

Work for Others

Sten Flodin, Swedish BioAlcohol Foundation
FIA-99-1458
This NREL Work For Others Funds-In-Agreement was completed in this period. Its goal was to perform pretreatment and fermentation testing at NREL on a mixed straw feedstock for the Swedish BioAlcohol Foundation and its partners, Weatherly Inc. and SWAN Biomass. The test plans were designed to obtain kinetic, yield and operating parameter values for a preliminary wheat, oat, and barley straw-to-ethanol facility design. The Statement of Work ultimately was comprised of seven tasks for NREL, including support of pretreatment testing, performance of fermentation testing and production of solid residue.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

- Industrial Partnerships presented at the Governor's Ethanol Coalition's first International Development Seminar on Fuel Ethanol in Washington, DC on December 14, 2001. This meeting was hosted by the state of Wisconsin on behalf of the Governor's ethanol coalition. NREL staff made a presentation on the current state of research and deployment at NREL on biomass-ethanol, particularly on the ESP.

- NREL staff attended and presented at an ethanol workshop in Yuma, CO, sponsored by Bryan and Bryan International. The meeting was well attended, with most of the attendees drawn from the farm sector, rural cooperatives, and rural development officials.

- NREL staff attended and presented at an ethanol workshop in Greensboro, NC, sponsored by DOE, the regional biomass program, and Ethanol Producers and Consumers (EPAC). The workshop was held in conjunction with the Governors' Ethanol Coalition meeting. The emphasis in NREL's presentations was to describe the most current lignocellulose-to-ethanol conversion technologies, the Biofuels Program's goals underlying the NREL Enzyme Sugar-Ethanol Platform, and the links between the emerging lignocellulose conversion technology and the existing corn ethanol industry.

Summary of Technical Achievements or Results

Communications

The primary focus of communications activity during the first half of FY 2002 was completion of a major redesign of the Biofuels Program Web site http://www.ott.doe.gov/biofuels/. The new design includes easier navigation, updated and new material, and "special audience" sections such as "For Researchers." This continues a trend of moving more information to the Web so that it can serve as a primary means of outreach. Also during the period, the calendar year 2001 issues of *Biofuels News* received a prestigious Award of Distinction from the Society of Technical Communicators. Publications during the period include:

- Biofuels News Fall 2001 — Farmer Attitudes toward Stover Collection
- Biofuels News Spring 2002 — The Enzyme Sugar Platform Project
- Biodiesel — Handling and Use Guidelines.

General Technical or Scientific Progress

Milestone Progress

C Milestone #377, More Effective Dissemination of Communications Products (6/30/02)

The intent of this milestone is to identify, for FY 03 implementation, ways to more effectively disseminate the communications products that we produce. This is essentially a brainstorming effort to lead to procedural commitment. One principal idea source will be the recommendations for reaching agricultural audiences developed by a subcontractor, Morgan and Meyers, during FY 01, many of which could be applied to all audiences. Activity thus far includes soliciting suggestions from DOE and Biofuels Program research staff, and seeking a final report from the consultants.

Progress Highlights and Issues

In keeping with Biofuels Program direction for FY 2002, we are shifting communications priorities in two ways. One is to toward greater reliance on the Web and more effective use of existing communications products, as indicated by Milestone #377 above. The other is toward increased emphasis on the Enzyme Sugar Platform Project in particular and industrial audiences in general. Other key external stakeholder audiences include the agricultural community, environmental interests, state government, and bioenergy/bioproducts. We are also taking on more in the way of general program functions in support of reporting and documentation requirements. For FY 2002, we have added additional staff resources to the cohesive communications team at NREL that continues to work well with Biofuels Program personnel and make communications an integral part of the Program.

Subcontractors

Morgan & Myers Inc., Gary Myers
ACE-1-31061-01
Reaching the Agricultural Community. Designing an Outreach Program for the Biofuels Program
4/30/2001-5/31/2002
The work for this subcontract has been completed. NREL is awaiting the final report from Morgan & Myers. The scope of work investigated the interrelationships among the agricultural community, production of agricultural residues and products suitable for feedstock for biomass ethanol production, biomass to ethanol technology, and the DOE National Biofuels Program.

Environmental and Energy Study Institute (EESI), Carol Werner
ACO-2-32004-01
Reaching the Environmental Community: Designing an Information Program for the NREL Biofuels Program
5/9/2002–5/8/2003
The subcontractor is to propose and carry out a program of outreach activities to environmental stakeholders about biomass ethanol production and use. The work will include the following tasks: Holding a series of briefings in the Washington, DC area for key policymakers and interest groups regarding the environmental impacts of ethanol production and use; Develop or provide an existing list of key energy and environmental stakeholders appropriate for the biofuels program; prepare and distribute quarterly or bimonthly, electronic or hardcopy, newsletters on environmental benefits and issues of biofuels to that stakeholder list.

Summary of Technical Achievements or Results

Process Development Unit/Data Acquisition And Control System (DACS) Maintenance

Milestone Progress/Completion

P Milestone #398, Integration of Pneumapress Controls (6/30/02)
The functional specifications for integrating the Pneumapress controls with the PDU control system are currently being defined by the pretreatment team. Physical design and installation of the system will begin as soon as the functional specification is complete.

P Milestone #399, Extend Functionality of PDU Task Application (9/30/02)
Steady progress has been made on refining existing capabilities of the PDU Task application. The pace of development on the application is expected to increase over the next few months to add tracking of equipment maintenance and instrument calibration.

P Milestone #400, Review Possible Replacements for G2 Process Control Software (9/30/02)
A preliminary review of possible replacements for the current G2 process control software has been made, however no strong candidates have been identified. A more thorough review of this issue needs to be completed.

Progress Highlights and Issues

Repairs and modifications to the PDU flash tank (MX-205) where completed. Damage to the flash tank agitator involved corrosion of the internal mechanisms of the agitator drive caused by the ingress of acid vapors through leaking seals. The damaged parts were repaired or replaced and a new seal design was employed to resolve the issue. Modifications to the agitator auger to improve the ability to process pretreated biomass at higher solids were also made while the system was dismantled.

The flash tank has functioned flawlessly during several pretreatment experiments and an extended run to produce pretreated corn stover for a 9000 L fermentation residue production experiment.

Subcontractors

Braconier
TCO-0-30039-01
Process Development Unit Mechanical Services
5/1/00-4/31/02
Major activities included installation of air, water, and steam piping for the Pneumapress filter system. It is planned to extend this subcontract through the third and final option year with additional funds.

RENEWABLE DIESEL FEEDSTOCKS AND PRODUCTION

Summary of Technical Achievements or Results

Fuel Production R&D

Four renewable diesel workshops were completed and the last one is in the final planning stages. The first in Sacramento, CA was cosponsored by the CEC, and addressed a variety of renewable diesel fuels. It had an attendance of 160. Media and other outreach was successful and some legislation at the state level has resulted from this effort. The second was in Seattle, WA and was jointly sponsored by the OR and WA Energy Offices and the Puget Sound Clean Cities Coalition. Support was also provided by the DOE Seattle Regional Office. 110 people attended and several small workshops in both WA and OR were initiated as a result OFD's initial investment. Several local organizations are planning to build biodiesel facilities in the Northwest. The third workshop, in Cedar Rapids, IA on October 3rd, was cosponsored by the IA Energy Office and the Iowa Soybean Board. Over 100 people attended including seven state legislators. The Iowa Energy Office is interested in follow on activities. The fourth workshop held on March 18th in Albany, NY, was cosponsored by the New York State Energy Research and Development Authority (NYSERDA) and was attended by about 110 people. The last workshop will be held in Cincinnati on April 13th. It will be cosponsored by the Energy Offices, Clean Cities, Soybean Boards, and DOE regional offices in Ohio, Kentucky, and Indiana.

The University of Idaho has isolated a mustard hybrid with extremely low polyunsaturated fatty acids (6%-10% total) and very low (<5%) saturated fatty acids. Oils high in monounsaturated fatty acids are considered optimal for biodiesel production as they will minimize NO_x increases and improve cold weather performance. This mustard variety will be cross-bred back into high glucosinolate hybrids to make a Brassica hybrid variety with perfect oils for biodiesel and strong economic potential. The average yield of field planted hybrids in four locations in the Pacific Northwest consistently exceeded 1 ton of seed per acre and averaged 1.4 tons per acre. The oil content of the seeds approached 40% on a regular basis. Seed yield increased by an average of 37% when insecticides were applied. Glucosinolate content averaged 160 μmoles/gram of defatted meal. Varieties were able to express or not express specific types of glucosinolates. Laboratory studies of the effectiveness of Brassica juncea and Sinapis Alba defatted meal on fungus gnats, Fusarium oxysporum, and nematodes have been conducted. The B. juncea performed better than the S. alba which may be a result of glucosinolates types, meal processing, or other unknown variables. Field trials with commercial partners are in the planning stages. A progress report was presented at the Annual Biodiesel Brainstorming Meeting in New Orleans in February 2002.

Brookhaven National Laboratory, on the behalf of NREL, showed that using B20 in home heating and industrial boilers could reduce NO_x emissions without system modifications. This research has been well received by the petroleum and heating oil

industries in the Northeast and has engendered industry sponsored research to bring the concept to the next stage in the commercialization process. The New England Fuels Institute and the Massachusetts Oilheat Council are jointly funding the second stage of this work. NYSERDA has also sponsored additional research in this area and is conducting field trials with a commercial heating oil vendor in NY. Equipment manufacturers have been involved through the Warwick School District Project. The Massachusetts Energy Office has provided $6,000 to Warwick to expand B20 activities through March 2003. All parties to this research area expect to commercialize B20 blends by fall of 2002. NREL, Brookhaven, and NYSERDA submitted a joint paper and a presentation was made at the Annual Biodiesel Brainstorming Meeting in New Orleans in February 2002.

The Puerto Rico biodiesel project has exceeded all expectations. University staff have submitted a patent idea disclosure for a novel processing technology. Widespread political support for using and producing biodiesel has emerged and several transportation and power demonstrations have begun with industry leaders. A phase II contract to build a PDU is under development.

General Technical or Scientific Progress

Milestone Progress/Completion

P Milestone #413, Renewable Diesel Workshops (7/31/02)
Continued educating customers and regulators about biodiesel fuels emissions, performance, fuel quality issues, availability, benefits, and costs. Four of the five have been completed and the last one will be completed by the end of April. This milestone is on track. In addition, two presentations to general groups have been done in Phoenix, AZ and another is planned in Portland, OR.

C Milestone #414, Mustard Project Stage Gate Review (7/31/02)
A Gate B review of the Mustard Project is conducted consistent with the Biofuels Program Stage Gate methodology including external stakeholders as reviewers. Since this is the first project review, stage placement needs to be confirmed. Review will confirm project stage by defining current status in the context of completion of Stage A activities and satisfaction of Gate B criteria. Future research needs and priorities are reflected in an acceptable Stage B work plan.

C Milestone #415, Life Cycle Inventory of Animal Fat and Grease Biodiesel (08/31/02)
A project to complete a life cycle analysis report on transforming animal fats and recycled greases to biodiesel. The completed report will be disseminated to stakeholders and will provide information on mass and energy balances, environmental releases, and greenhouse gas emissions to enable sound policy decisions. A draft industry survey is being developed and will be submitted to rendering industry stakeholders in April. Process engineering and boundary definition is underway. The chapters on integrated and independent rendering have been started. Work on meat animal production has begun and is being supported by the University of Kansas and Dr. Richard Nelson on a cost-share basis. This project is on track.

P Milestone #416, Trap Grease Project (9/30/02)
The purpose of this project is to provide information to water treatment industries and biodiesel producers about the benefits of diverting trap grease and using a low value feedstock for biodiesel. The subcontractor prepared a report describing a feasibility analysis of diverting trap grease from water sanitation plants to biodiesel and the results of a pilot scale demonstration. This contract began at the start of March and is anticipated to be delivered on time.

P Milestone #417, Educational Tools Project (9/30/02)
This project will teach future biodiesel stakeholders how to make high quality biodiesel through courses offered to small scale producers and avoid the introduction of poor quality biodiesel from under capitalized producers that could destabilize the growing biodiesel industry. Introduction to Biodiesel curriculum will be ready by September 30, 2002. The other three courses will follow in FY 2003. A steering committee is being developed.

Progress Highlights and Issues

A contract for developing course work for four biodiesel college level courses with Iowa State was placed in December 2001 and the first course should be ready by fall 2002.

A contract with Ocean Air Environmental and Glycerin (a leading multifeedstock biodiesel producer) was placed in February 2002 to demonstrate the commercial potential of producing biodiesel from trap and sewage greases. A consortium of sewage plant operators and waste grease haulers and the biodiesel firm has been created. The partners are enthusiastic about the commercial potential of the project and are working with local legislators to tighten up trap grease regulations to improve the environment.

Phase III contract with University of Idaho has been initiated. It involves some continued breeding efforts ($106,000), but focuses most of the newest research on a market assessment ($65,000), pesticide trials, pesticide release efficiency tests, and commercial partnerships for field testing ($160,000). A stage gate review of the project is planned for July.

A modification to the Oxidative Stability contract with Southwest Research will be implemented to refine test methods for B100 and redo the time and temperature test matrix.

Collaborative Activities

The Fats and Protein Research Foundation has been instrumental in developing a survey instrument for the Life Cycle Inventory project. They will provide cover letters, a mailing list, and other support at their annual board meeting in April 2002.

The University of Idaho staff on the mustard project have submitted the following proposals to USDA:

- Application of Brassica Meal for Disease Control and Improved Nitrogen Fertility in Organic Farming Systems. Co-investigator of a joint proposal with J. Johnson-Maynard and L.-M. Dandurand. Organic Farming Research Foundation, $27,770.

- MustardSeed Meal as a Soil Amendment for Weed Control and Improved Nitrogen Fertility in Organic Farming Systems. Principal investigator of a joint proposal with J. Johnson-Maynard and D. Thill. USDA/CSREES, $247,591.

The University of Puerto Rico (UPR) Project is a collaborative effort with the DOE Office of Diversity and Economic Development and OFD. UPR is the primary contractor and a private engineering firm Panzardi-ERM (PERM) is a lower tier subcontractor. Phase I was funded with $100,000 from each DOE office and Phase II will be funded in a similar manner in FY2002.

Phase I is nearing completion although the demonstrations requested in Task 5 have been delayed because of delays with MOUs and local co-funder's funding schedules. Seven local demonstrations have been organized, half with power and half with transportation uses. Three reports have been submitted: (1) Task 3 – Develop Laboratory Expertise on Biodiesel Processing Technologies and Develop Data for Engineering Designs; (2) Task 5 - Identify Industry Partnerships for Biodiesel Production and Marketing and Demonstrations; and (3) Task 1 – Resource Assessment.

Phase I has exceeded all expectations for success. UPR and PERM have developed a sizable pool of local chemists and engineers with growing expertise in biodiesel production technology and marketing. Numerous government agencies and local private firms have joined with the project coordinators to expand on the original contract with demonstrations, funding, and other resources.

UPR collaborative activities for Phase II have included:

- Senor Oscar Sebo Serrano from the Puerto Rico Industrial development Corporation met with UPR and ERM staff to discuss co-sponsoring phase II PDU activities.

- Three Municipal Recycling Directors (Rincón, Isabela, and Moca) met with UPR to discuss providing subsidized labor for feedstock collection. This and the two Isabela meetings were organized by David Muñoz. He is coordinating all the activities related to interacting with West Coast (of Puerto Rico) municipalities.

- The president of Environmental Power, Eric Rodreguez and their environmental engineer Alex Flores met with UPR staff to discuss Phase II participation as potential cost-share partners.

Cross-Program Research Activities

Most cross-program research activities have been transferred to R. McCormick. A working meeting of E-diesel stakeholders was held in Chicago in October 2001 and attended by K.S. Tyson. The working group decided to form an industry trade association to handle some of the regulatory issues related to commercializing the fuel.

NREL worked with OFD to submit a Small Business Innovative Research idea to the process. Other support activities included developing a strategic overview of the project for the new DOE management.

Technology Transfer/Industry Outreach

The biodiesel fact sheet DOE/GO-102000-1048 was updated and reprinted as DOE/GO-102001-1449 in February 2002. "Biodiesel Handling and Use Guidelines," NREL/TP-580-30004 was printed in September 2001 and has been very popular with fleet operators. K.S. Tyson provided input for a new Clean Cities Biodiesel Fact sheet and to several other DOE publications and Web sites.

Subcontracts

Iowa State University
ACO-2-31056-01
"Biodiesel Educational Tools" was placed December 2001. Four college-level courses will be developed to improve the quality and business practices of small biodiesel producers in the U.S. The first course, Introduction to Biodiesel, should be ready to preview by Fall 2002.

Ocean Air Environmental Services
NCI-2-31097-01
"Feasibility and Demonstration of biodiesel Production from Recovered Trap Grease" was placed February 2002. A feasibility study documenting the benefits of trap grease recovery for waste water treatment plants and the economics of converting trap grease to biodiesel will be provided. The biodiesel firm will demonstrate the feasibility of converting trap grease into fuel grade biodiesel. This contract should be complete by October 2002.

Brookhaven National Laboratory
DAR-1-31053-01
"Heating Oil Assessment" subcontract was completed and a final report provided. A technical paper will be presented in an oil heat trade journal in April 2002.

University of Idaho
XCO-9-29095-01
"Mustard Crops for Biodiesel Phase II" made considerable progress hybrids vigor and design, chemical characterization of meals and oils, and laboratory pesticide testing.

Field tests with suboptimal meal with commercial industry partners shall begin in 2002. Phase III contract modifications should be complete by the end of March 2002.

Colorado School of Mines
XCO-0-30088-01
"NO_x Solutions for Biodiesel" is in the process of close out.
Colorado School of Mines
ACG-8-17106-01
"Health Related Emissions From Biodiesel…" One final report and one draft final report has been received. The most recent draft characterizes basic combustion chemistry of biodiesel fuels. It is in final review and a final is expected by September 2002. This contract has been extended to accommodate the final review period.

Southwest Research Institute
AXE-9-29057-01
"Biodiesel Emissions from Locomotives" has been completed. A final report was received in December 2001 "Evaluation of Biodiesel Fuel in an EMD GP38-2 Locomotive." NREL will publish this report in the near future.

Southwest Research Institute
ACG-7-17066-01
"Oxidative and Thermal Stability Testing Methods for Biodiesel" was extended through May 2002 to allow completion of the work.

Pacific Biodiesel
ACO-9-29045-01
"Composition Analysis of Waste Grease" recently submitted 50% of the composition data. The literature review was resubmitted and accepted. The contract modification to descope and close the contract out was signed in March 2002.

Advanced Fuel Solutions
XCO-0-30044-01
"Petroleum Infrastructure Development" has been modified to add $30,000 to support biodiesel education with the heating oil industry and support the Warwick project. A new task to examine additive issues in biodiesel blends was added.

Proforma Systems
TXL-9-29031-01
"Biodiesel Process Engineering and Economics" is being cancelled for nonperformance. A unilateral modification is in progress and should be complete by the end of March 2002.

University of Puerto Rico
ACO-1-30119-1
"Grease Biodiesel for Puerto Rico" OFD and ED have jointly funded a project with the University of Puerto Rico Mayaguez and Panzardi ERM. Phase I is near completion and Phase II planning is underway. The contract should be modified by May 2002 to add $200,000 for ongoing R&D and to build a PDU. The City of Caguas has cost-

shared $20,000 toward a demonstration and the Energy Administration Office is cost-sharing another demonstration with an additional $25,000.

University of California Davis
ACG-8-17087-01
"Chemical and Bioassay Analyses of the Particle and Semi-Volatile Emissions from SME and Biodiesel Blended Fuels" has submitted two final reports and is in the process of preparing a professional paper for a industry trade journal. One report has been edited and will be published by the end of March 2002 and the other will be published soon after. The professional paper is expected by September 2002, after which the contract will be closed out.

Institute of Gas Technology
ACG-7-15177-02
"Biodiesel Project: Multi-Feedstock Biodiesel Testing, Production and In-Use Demonstration" submitted a final report which has been edited and is ready to be published by the end of March 2002. This contract is in closeout.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

With the assistance of numerous co-sponsors NREL organized the following workshops:

- Renewable Diesel Fuels: The Flexible Options, Sacramento, CA. Sept 25, 2001.
- Greening Ahead of the Curve: Meeting the Challenge with Renewable Diesel Fuels, Seattle, WA, Sept 27, 2001.
- From the Field to the Fuel Tank: The Biodiesel Option, Cedar Rapids, IA, Oct 3, 2001.
- Moving Towards Energy Independence: Biodiesel Workshop, Albany, NY. March 18, 2002.
- Upcoming: A Biodiesel Odyssey: In the Heartland, Erlanger, KY, April 11, 2002.

S. Tyson presented an overview of biodiesel fuel properties, performance characteristics and emissions, including feedstock supplies, their impacts on fuel properties and the mustard project at each of these. Teresa Alleman also presented at CA and WA on multifeedstock emissions and NOx control.

Other presentations to the General Public

Montana Biofuels Roundup, October 11, 2001. Presented highlights of the Mustard Project

Great Lakes Canola Growers Association, Gaylord, MI, October 18, 2001, Presented Program Overview and Highlights of the Mustard Project.
Maricopa County Biodiesel Workshop, Tempe AZ, October 24, 2001. Presented Highlights of the Renewable Diesel Program and the Mustard Project.

South Coast Air Quality Management District, Diamond Bar, CA, October 25, 2001. Presented detailed discussions of biodiesel emissions, engine performance and air toxics to senior staff.

Biofuels Seminar, Hosted by the Maricopa County Environmental Services Dept, SBEAP and the City of Phoenix, Phoenix, AZ, February 5, 2002. Presented general technical biodiesel information and highlights of the mustard project.

Attended the OFD Strategic Planning Meeting in Harbourtowne, MD on Nov. 13-15, 2001.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

Scientific Journals: Papers Accepted for Publication

Roger J. McDonald and C.R. Krishna, BNL Oilheat Research, and Shaine Tyson, NREL *Biodiesel Goes Off-road...Brookhaven National Laboratory Studies Biodiesel as Heating Oil Supplement.* To be published in the National Oilheat Research Association, April 15 Conference Report.

Posters

“Mustard Hybrids for Low-Cost Biodiesel and Organic Pesticides” K. Shaine Tyson and Jack Brown (Univ. of Idaho), Poster at the AICHe Critical Issues Series: Energy and a Sustainable Plant, New Orleans, LA, March 10-14, 2002.

Technical Reports

Tyson, K.S. *Biodiesel—Clean, Green Diesel Fuel,* September 2002. NREL/GO-102002-1449. Updated.

Robert L. McCormick, Javier R. Alvarez, and Michael S. Graboski. January 2002. *NOx Solutions for Biodiesel.* NREL/TP-510-31465.

Norman Y. Kado and Paul A. Kuzmicky. December 2000. *Bioassay Analyses of Particulate Matter From a Diesel Bus Engine Using Various Biodiesel Feedstock Fuels*. NREL/SR-510-31463.

Michael S. Graboski, Robert L. McCormick, Teresa L. Alleman, and Andrew M. Herring. January 2002. *The Effect of Biodiesel Composition on Engine Emissions from a DDC Series 60 Diesel Engine.* NREL/SR-510-31461.

Mustafa E. Tat and Jon H. Van Gerpen. February 2002. Measurement of Biodiesel Speed of Sound And Its Impact on Injection Timing. NREL/SR-510-31463

John A. Kinast. February 2002. *Production of Biodiesels from Multiple Feedstocks and Properties of Biodiesels and Biodiesel/Diesel Blends.* NREL/SR-510-31460.

C. R. Krishna. December 2001. *Biodiesel Blends in Space Heating Equipment*. BNL-68852.

Summary of Technical Achievements or Results

Renewable Diesel Technical Barriers R&D

Discussions with fuel injection equipment and engine manufacturers regarding use of biodiesel have revealed several concerns about compatibility with fuel system components. These companies have found, through a survey of their customers, that while most users have no technical problems when using 20% biodiesel blends with conventional diesel, a small number of customers experience catastrophic fuel pump failure. The reason for this is not known at present, but is likely to be caused by poor fuel quality. Our current hypothesis is that some biodiesel has poor oxidative stability and that oxidation may lead to the presence of components that can cause pump failure. Discussions and data gathering relevant to this problem are continuing, and a plan for testing to determine if pump failure can be caused by oxidation will be developed. Note that development of test methods for measuring biodiesel oxidative stability is ongoing under the Renewable Diesel Feedstocks and Production R&D Project.

NREL has worked with the Renewable Fuels Association (RFA) and industry representatives to facilitate the formation of an industry consortium to perform R&D on ethanol-diesel blends (e-diesel). An initial meeting was held in Chicago on October 9, 2001. Attendees for DOE were G. Santos-Leon and N. Zerbi and for NREL were R. McCormick, S. Whitacre, and K.S. Tyson. Representatives from ethanol producers, corn growers, e-diesel additive suppliers, fuel additive suppliers, and state agencies attended. The outcome of the meeting was a consensus vote to form an e-diesel consortium under RFA. NREL has provided support to RFA in forming this consortium. Following the RFA meeting in San Diego on March 1, 2002 the members voted to formally constitute their organization as a subset of the RFA. Current members include RFA, National Corn Growers Association, Illinois Department of Commerce and Community Affairs, AAE, Inc., Akzo-Nobel, Betz Dearborn, Lubrizol, Octel-Starreon, Pure Energy Corporation, ADM, Cargill, and Williams Bio-Energy. Discussion of research priorities indicated that fuel pump compatibility and fuel flammability were top priorities. Plans for NREL to perform R&D to address these issues are being developed.

General Technical or Scientific Progress

Milestone Progress/Completion

C Milestone #419, Advanced Engine and Emission Controls Testing, (5/31/02)
This milestone is on track for completion in May. Discussions with the Fuels Utilization Program APBF-DEC project group have been initiated. The current contracts are being evaluated to determine if contract modification to perform this work is a reasonable option, or if a new contract will be required.

C Milestone #420, Fuel Injection Equipment (FIE) Manufacturers Outreach, (8/31/02)
This milestone is on track for completion in August. Contacts have been initiated to determine FIE manufactures' issues with biodiesel. Visits or teleconferences with these companies will be scheduled during April and May.

P Milestone #422, E-Diesel Properties and Materials Compatibility, (9/30/02)
OFD and E-diesel industrial stakeholders approved a preliminary plan for this project on March 1. Detailed scope was developed during March.

P Milestone #423, Web-Based Outreach, (7/31/02)
Web pages have been updated with new information. This is being reviewed and additional modifications may be made if necessary.

P Milestone #421, Renewable Diesel Strategic Plan, (5/15/02)
This internal project made good progress in January and February and is anticipated to remain on schedule.

Progress Highlights and Issues

Cross-Program Research Activities

The Renewable Diesel Technical Barriers R&D Project is being performed in collaboration with the Fuels Utilization Program's Renewable Diesel and Biolubricants Project, which is also managed by NREL. The Fuels Utilization Program and the Office of Heavy Vehicle Technologies (OHVT) are providing input for the Renewable Diesel Strategic Plan. OHVT has also requested development of an R&D plan for renewable diesel fuels utilization R&D that will be highly coordinated with efforts in the OFD Renewable Diesel Program. This plan should be completed by July 2002.

Subcontracts

A solicitation for a project to examine fuel pump wear and seal compatibility for e-diesel is planned for early in the second half of this year.

An Oxygenated Diesel Particulate Matter Emission Project is slated for an August, 2002 start date. An RFP has been submitted and reviewed.

Scientific Publications, Presentations, and Other Activities

General Presentations/Travel

October 9, Chicago, IL, Initial meeting of E-diesel consortium.

November 6, Washington, DC, National Biodiesel Board Meeting. Presentation on Renewable Diesel Program structure.
November 6, Washington DC, NREL review of several OFD programs. Presentation on Renewable Diesel Technical Barriers R&D Project.

January 23-24, New Orleans, LA, National Biodiesel Board Research Brainstorming Meeting. Presentations on OFD Renewable Diesel Program and on use of fuel additives to reduce biodiesel NOx emissions.

February 28-March 1, San Diego, CA, Renewable Fuels Association National Ethanol Conference. Presentation on e-diesel.

Scientific Meetings: Papers/Posters Presented or Recently Accepted for Presentation

January 23-24, New Orleans, LA, National Biodiesel Board Research Brainstorming Meeting. Presentation entitled "Fuel Additive and Blending Approaches to Reducing NOx Emissions from Biodiesel"

February 28-March 1, San Diego, CA, Renewable Fuels Association National Ethanol Conference. Presentation entitled "Technical Barriers to Use of Ethanol in Diesel Fuel"

May 6-9, Reno, NV, Society of Automotive Engineers Fuels and Lubricants Meeting. Presentation entitled "Fuel Additive and Blending Approaches to Reducing NOx Emissions from Biodiesel" accepted.

Scientific Journals: Papers Accepted for Publication

McCormick, R.L., Alvarez, J.A., Graboski, M.S., Tyson, K.S., Vertin, K. " Fuel Additive and Blending Approaches to Reducing NO_x Emissions from Biodiesel" Society of Automotive Engineers Technical Paper No. 2002-01-1658 to be published.

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13. ABSTRACT *(Maximum 200 words)*

Management report for the first half of fiscal year 2002. The report summarizes the achievements and activities of the Pretreatment Technology, Improved Cellulase Enzymes, Strain Development, Enzymatic Process Development, Lignin Conversion to Fuels, Collaborative Industrial Process Development, Process Engineering and Development, and Renewable Diesel Project teams.

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